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Problem Formulation for Human Health Risk Assessments of Pathogens in Land-applied Biosolids

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LIST OF ABBREVIATIONS

CFR	Code of Federal Regulations
HPC	heterotrophic plate counts
ICC-PCR	integrated cell-culture PCR
NRC	National Research Council
PCR	polymerase chain reaction
PSRP	process to significantly reduce pathogens
RT-PCR	direct reverse transcriptase PCR
U.S. EPA	United States Environmental Protection Agency

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1. INTRODUCTION

In January 2004, the United States Environmental Protection Agency (U.S. EPA) released a final action plan for setting new priorities for the biosolids program, which included the Agency's response to the National Research Council (NRC) report entitled *Biosolids Applied to Land: Advancing Standards and Practice* (NRC, 2002). This report is an important step in the Agency's response because it addresses the development of a problem formulation and analysis plan relating to uncertainties associated with conducting quantitative microbial risk assessments on land-applied biosolids. This report summarizes the existing literature (Appendix A); defines critical pathogen stressors; develops conceptual models linking the most likely stressors, pathways and health responses of concern; evaluates the overall quality and utility of available risk assessment data, tools and methodologies; and develops an analysis plan which identifies the research and methods required for providing a scientifically defensible risk assessment relevant for U.S. EPA's decision needs.

"Problem formulation is a systematic planning step that identifies the major factors to be considered in a particular assessment" (U.S. EPA, 2003a). It was developed for ecological risk assessment and was subsequently adopted for cumulative human health risk assessments (U.S. EPA, 1998, 2003a). The principal products of problem formulation are a conceptual model and an analysis plan (U.S. EPA, 2003a).

This generic problem formulation should serve two audiences. First, assessors who must assess risks to human health from land-applied biosolids can use this generic problem formulation as a basis for developing their own problem formulations. It can serve as a template, an information source and an introduction to the relevant literature.

Second, the research needs identified in this report can be used by researchers and research planners to select and prioritize research projects related to pathogens in biosolids. It can also help researchers to understand how to design their studies so as to generate results that will be relevant to risk assessment.

2. STRESSOR CHARACTERIZATION

Stressors are chemical, physical or biological agents that may adversely affect human health or other assessment endpoints. The description of stressors is a necessary precursor to developing conceptual models, especially for risk assessments of a complex substance like biosolids. U.S. EPA (1998) describes several questions that a stressor characterization for an ecological risk assessment should answer. These points are modified for human health risk assessments for pathogens.

1. What is the source of the pathogens?
2. What is the spatial extent of the source?
3. What types of stressors are present: physical, chemical or biological?
4. What are the modes of action of the stressors?

Essentially, sources and stressors must be characterized well enough to inform decisions about the conceptual models and exposure pathways within them that are needed to characterize all reasonable exposure scenarios. For example, pathogens in bioaerosols have different fates from those that remain in biosolids-amended soil particles, and the problem formulation should describe these differences.

This report focuses on pathogens and endotoxins originating in biosolids. In addition to descriptions of microorganisms in biosolids, the assessor should include aspects of the biosolids matrix that affect pathogenicity and dimensions of the source that affect how exposure is modeled or monitored. Studies of untreated manures are beyond the scope of this report.

This chapter describes the biosolids source, including the components of the mixture, the extent of the source, the matrix, the Class B treatment process, site restrictions and vector attraction reduction options. Following the description of the source is pertinent information about bacterial, viral, protozoan and helminth pathogens, as well as endotoxins that may be present in biosolids and may cause adverse effects to human health.

2.1. SOURCE

Approximately 3.4 million tons of biosolids, dry weight, are land-applied annually to farms, forests, rangelands, mine lands and other land use types (Pepper et al., 2006; NRC, 2002). These soil amendments have nutrients for plant growth as well as components that improve physical properties of soils. The U.S. EPA did not use the term biosolids in the Part 503 rule, but U.S. EPA (1995) defines biosolids as “the primarily organic solid product yielded by municipal wastewater treatment processes that can be beneficially recycled” as soil amendments. The NRC’s definition of biosolids is “sewage sludge treated to meet the land-application standards in the Part 503 rule or any other equivalent land application standards” (NRC, 2002). Pathogen standards are technologically based requirements “aimed at reducing the presence of pathogens and potential exposures to them by treatment or a combination of treatment and use restrictions” (NRC, 2002).

Biosolids are a complex mixture that contains organic and inorganic compounds and organisms from wastewaters of households, commercial and industrial facilities, as well as compounds added or formed during wastewater treatment processes (NRC, 2002). Inorganic and organic contaminants in biosolids are also described in NRC

(2002) and may include metals, trace elements, PCBs, dioxins, pharmaceuticals, surfactants and other contaminants.

2.1.1. Spatial Extent of Source

Risk assessors need to characterize the areal extent of biosolids application or storage that is the subject of the risk assessment. Biosolids may be localized or more diffuse sources of infectious microbes. Pathogen transport models may be specific to the spatial extent of the source. Large piles of biosolids that serve as temporary storage before placement can represent continuous, localized sources of pathogen-containing bioaerosols (described below) (Dowd et al., 2000). Similarly, bioaerosols can be created during the transport of biosolids from one location to another at a site, during the 'front-end loading' or "shoveling" of biosolids from one pile to another, or from the lifting of biosolids-amended soil particles by strong winds (Pillai, 2007). Areas of application may be large fields or more localized windrows. If the risk assessment is intended to estimate cumulative risk, then biosolids application in adjacent fields over time may be pertinent. At the extreme, a risk assessment may address the entire area treated with biosolids nationally or by state.

2.1.2. Reproduction

In addition to providing physical reservoirs of pathogens, biosolids and biosolids-amended soils can serve as sources of additional pathogens as some of the organisms reproduce (Zaleski et al., 2005a). Evidence about reproduction or lack of reproduction of particular species is important information for the conceptual models.

2.1.3. Matrix

Four principal biosolids-containing matrices are possible sources of pathogens: liquid biosolids, solid biosolids, biosolids-amended soil and bioaerosols created from biosolids. Bioaerosols are of particular interest in this problem formulation.

1. *Liquid biosolids.* Liquid biosolids are the texture of muddy water and usually contain 2-8% solids (Paez-Rubio et al., 2007). They are expensive to transport.

2. *Solid biosolids*. Biosolids cake (usually 20-30% solids content) (Paez-Rubio et al., 2007) is dewatered biosolids with the texture of a wet sponge (Virginia Department of Health, 1999).

3. *Biosolids-amended soil.* Over repeated applications, biosolids-amended soil has different physical properties from soil alone. The altered physical properties of soil include increased water holding capacity, water infiltration and stability of soil aggregates (University of Washington, 2002).

4. *Bioaerosols.* Bioaerosols are aerosolized biological particles that vary from 0.02 to 100 µm in diameter. They are formed when dewatered biosolids are loaded into application equipment or when liquid and dewatered biosolids are spread onto land (Paez-Rubio et al., 2007). The following information comes from references in Pillai and Ricke (2002) and Pillai (2007). The size, composition and concentration of microbial populations comprising aerosols vary with biosolids source, method of application and meteorology and other environmental conditions at the biosolids application site. Bioaerosols generated from water sources (e.g., liquid biosolids) usually have a thin layer of moisture surrounding clusters of microorganisms. Bioaerosol particles have a net charge that depends on the source characteristics and can affect deposition rates. Factors that control bioaerosol transport include the size, density and shape of particles or droplets, as well as wind speed, relative humidity and temperature. When some aerosolized bacteria are exposed to high relative humidity, they sorb water, which protects the cells from inactivation by ultraviolet light (Peccia et al., 2001).

2.1.4. Class B Treatment

A description of the sewage sludge treatment process provides risk assessors with information about the potential pathogen content of biosolids. Treatment methods

are intended to reduce the volume and organic content of biosolids and to reduce the number of pathogens, but to retain beneficial properties for fertilization and other soil amendment and land reclamation purposes (NRC, 2002). The Part 503 rule defines two categories of biosolids: Class A biosolids, which have no detectable concentrations of pathogens, and Class B biosolids, which have detectable concentrations of pathogens (U.S. EPA, 1993). This report focuses on Class B biosolids, which are defined by a combination of treatment requirements and site restrictions. The treatment of these biosolids must meet one of three criteria: fecal coliform count of less than 2×10^6/gram of dry solids at the time of disposal, treatment by a process to significantly reduce pathogens (PSRP), or treatment by a process equivalent to PSRPs. Five processes in the Part 503 Rule were determined to be PSRPs, based on their resulting fecal coliform concentrations less than 2×10^6/gram of dry solids and their ability to reduce *Salmonella* and enteric virus levels by a factor of 10 (U.S. EPA, 1999):

1. Aerobic digestion at specific combinations of time and temperature,
2. Air drying for three months, with average ambient daily temperatures above freezing for at least two months,
3. Anaerobic digestion for specific combinations of time and temperature,
4. Composting for specific combinations of time and temperature and
5. Lime stabilization to give a pH greater than 12 after 2 hours of contact.

Fecal coliforms are enteric bacteria that are used as indicators of the likelihood of the presence of bacterial pathogens. *Salmonella* species are human pathogens. In this problem formulation, it is assumed that treatment requirements and site restrictions

meet standards. If sewage sludge is dewatered, thickening agents such as ferric chloride, lime or polymers are added (NRC, 2002).

2.1.5. Site Restrictions

Site restrictions also provide information about the content of biosolids to which humans are exposed, because pathogens attenuate over time. Site restrictions are required to reduce contact with Class B biosolids until environmental exposures such as heat and desiccation have decreased concentrations of bacterial, viral and helminth pathogens to below detectable concentrations equivalent to those in Class A biosolids (NRC, 2002). Natural attenuation also incorporates biological factors such as competition, predation, hyperparasitism (growth of a secondary microorganism in or on the primary pathogen or parasite) and antibiosis (Smith et al., 2005a). Site restrictions to public access, grazing and harvesting are included (Table 1).

2.1.6. Vector Attraction Reduction

The Part 503 rule requires that one of ten management options be used to control disease vectors. These are described in detail in the rule and in NRC (2002): volatile solids reduction, specific oxygen uptake rate, anaerobic bench-scale test, aerobic bench-scale test, aerobic process for compost, pH adjustment, drying without primary solids, drying with primary solids, injection and incorporation. The first eight options are process-based options, the first five of which are intended to contribute to long-term stabilization through the degradation of putrescible organics. Injection of

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TABLE 1 Site Restrictions for Class B Biosolids (Copied from NRC (2002), Adapted from 40 CFR 503.32[b][5])
Food crops with harvested parts that touch the biosolids/soil mixture and are totally above the land surface shall not be harvested for 14 months after application of biosolids.
Food crops with harvested parts below the surface of the land shall not be harvested for 20 months after application of biosolids when the biosolids remain on the land surface for four months or longer prior to incorporation into the soil.
Food crops with harvested parts below the surface of the land shall not be harvested for 38 months after application of biosolids when the biosolids remain on the land surface for less than four months prior to incorporation into the soil.
Food crops, feed crops and fiber crops shall not be harvested for 30 days after application of biosolids.
Animals shall not be grazed on the land for 30 days after application of biosolids.
Turf grown on land where biosolids is applied shall not be harvested for one year after application of the biosolids when the harvested turf is placed on either land with a high potential for public exposure or a lawn, unless otherwise specified by the permitting authority.
Public access to land with a high potential for public exposure shall be restricted for one year after application of biosolids.
Public access to land with a low potential for public exposure shall be restricted for 3 days after application of biosolids.

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biosolids and incorporation within 6 hours of application are considered physical barriers to vector attraction.

2.2. PATHOGENS

A variety of bacterial, viral, protozoan and helminth pathogens may be present in Class B biosolids. Risk assessors should consider and list the range of possible pathogens in the problem formulation, though it may be necessary to focus on only a limited number. Many of these organisms and the diseases they cause are summarized in Table 2. Researchers who list principal pathogens of concern in sewage sludge and/or biosolids do not always list the same organisms (NRC, 2002; Gerba and Smith, 2005; Pepper et al., 2006; Epstein, 2006; Yanko, 2005). As biological stressors, pathogens can multiply, and many can reproduce outside of the host organism under favorable environmental conditions. The types and levels of pathogens in biosolids are determined by the incidence of infection within a community and the type of treatment process (Straub et al., 1993). The biosolids matrix (i.e., whether humans are exposed to biosolids, biosolids-amended soil, bioaerosols, or biosolids particles in water) may affect the fate of pathogens, and therefore determine exposure.

2.2.1. Bacteria

2.2.1.1. *Salmonella*

All serotypes of this genus are pathogenic to humans and cause symptoms ranging from mild gastroenteritis to severe disease and death. In the U.S., salmonellosis is mainly due to foodborne transmission because the bacteria found in

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TABLE 2

Example Pathogens of Potential Concern in Sewage Sludge and Biosolids

Class	Organism	Disease or Symptoms
Bacteria	*Listeria monocytogenes*	Meningitis, encephalitis, septicemia, intrauterine or cervical infections with abortion
	Helicobacter pylori	Stomach ulcers, gastritis, increased risk of stomach cancer
	Campylobacter jejuni	Gastroenteritis
	Pathogenic *Escherichia coli*	Gastroenteritis, hemolytic uremic syndrome
	Shigella spp.	Bacillary dysentery
	Salmonella spp.	Salmonellosis (food poisoning), typhoid/paratyphoid fever
	Yersinia spp	Yersiniosis (gastroenteritis)
	Legionella spp.	Severe respiratory illness, mild flulike illness
Viruses	Astroviruses	Gastroenteritis
	Rotaviruses	Gastroenteritis
	Caliciviruses	Gastroenteritis
	Adenoviruses	Respiratory diseases, gastroenteritis
	Hepatitis virus A-E	Infectious hepatitis, liver inflammation, hepatic cancer
Helminth Parasites	*Taenia spp.*	Nervousness, enteric distress, abdominal pain, anorexia, insomnia
	Ascaris lumbricoides	Digestive disturbances, abdominal pain, transitory liver and lung disease

Table 2 (cont.)

Class	Organism	Disease or Symptoms
Helminth Parasites (cont.)	*Trichuris spp.*	Gastrointestinal distress, anemia
	Toxicocara canis	Fever, abdominal discomfort, neurological symptoms
Protozoan Parasites	*Cryptosporidium parvum*	Diarrhea
	Giardia lamblia	Fever, diarrhea
	Cyclospora	Diarrhea, nausea, vomiting and abdominal cramps
	Microsporidia	Diarrhea
	Entamoeba histolytica	Dysentary, colitis
	Balantidium coli	Diarrhea, constipation, abdominal pain

Sources: Gerba and Smith (2005), Epstein (2006), NRC (2002), Pepper et al. (2006) and Bowman and Fayer (2005).

beef and poultry are able to grow in foods (Pepper et al., 2006). As of 1998, there was no known association of biosolids with foodborne outbreaks of *Salmonella* (Yanko, 2005). However, *Salmonella* can apparently grow in biosolids under some conditions (Zaleski et al., 2005a). Because of this potential for growth, Pepper et al. (2006) argue that *Salmonella* are the bacteria of greatest concern in Class B biosolids. They are the 40 CFR 503 bacterial pathogen indicators for biosolids quality,

2.2.1.2. *Escherichia coli O157:H7*

Escherichia coli is found in the intestinal tract of humans and most warm-blooded animals, and most strains are not pathogenic. However, several strains can cause gastroenteritis. The greatest concern in the U.S. is enterohemorrhagic *E. coli* of the serotype O157:H7 (Pepper et al., 2006). The organism has been spread in contaminated drinking water, through recreational water exposure and food (Yanko, 2005; Pepper et al., 2006). Cattle are the most significant source of exposure, but the organism has been detected in biosolids (Lytle et al., 1999; Pepper et al., 2006).

2.2.1.3. *Campylobacter jejuni*

This pathogen is the principal cause of bacterial diarrheal illness in the U.S. Food is the major source of infection. Little research has been conducted to investigate the occurrence of *Campylobacter* in sewage sludges, biosolids, or the environment (Yanko, 2005), though a few studies of raw and treated sludge are reviewed in Pepper et al. (2006).

2.2.1.4. *Shigella Spp.*

Bacteria of this genus are closely related to *E. coli.* The bacteria are frequently found in water contaminated with human sewage and are transmitted by the fecal-oral route. Salads, raw vegetables, milk and dairy products and poultry sometimes are polluted with *Shigella* (Pepper et al., 2006). The pathogen has a low infectious dose. *Shigella* does not survive well in the environment or after treatment of biosolids. Therefore, they are unlikely to be a significant problem (Pepper et al., 2006).

2.2.1.5. *Yersinia Spp.*

These bacteria cause gastroenteritis with diarrhea or vomiting, fever and abdominal pain. *Yersinia enterocolitica* has been detected in environmental sources such as ponds and lakes, though the major source of infection in the U.S. is pork products (Pepper et al., 2006). Waterborne outbreaks have also occurred. In Japan infections of *Y. pseudotuberculosis* from contaminated water and foods have been reported. The bacterium has been detected in raw, digested and dewatered biosolids (Straub et al., 1993), but little information is available about background levels or survival in soils or waters (Pepper et al., 2006).

2.2.1.6. *Listeria montocytogenes*

This bacterium causes foodborne diseases, primarily in immunocompromised people such as pregnant women. It can cause encephalitis, meningitis and intrauterine or cervical infections (Epstein, 2006). *L. montocytogenes* has been detected in activated and anaerobically digested biosolids (Watkins and Sleath, 1981; DeLuca et al., 1998). The bacterium is widespread in the environment (Yanko, 2005).

2.2.1.7. *Helicobacter pylori*

This bacterium is the principal cause of stomach ulcers and is associated with increased risk of stomach cancer. *H. pylori* may be the most common cause of bacterial infection in humans (up to 90% of some populations are infected, Epstein 2005), though rates of infection are decreasing (Yanko, 2005). The source of many infections is vegetables irrigated with untreated wastewater (Brown, 2000). The digestive tract of humans is apparently the main reservoir of *H. pylori* (Yanko, 2005). Whether *H. pylori* is present in Class B biosolids is unknown (Pepper et al., 2006).

2.2.1.8. *Legionella*

Infections with *Legionella* can result in a life-threatening respiratory illness, Legionnaires' Disease, especially in immunocompromised people or the elderly, or a mild illness called Pontiac Fever. Outbreaks of *Legionella* usually occur through airborne transmission of bacteria from hot water in building cooling towers or other aerosolizing devices (Yanko, 2005). High concentrations have been measured in biosolids at a food industry sewage treatment plant where workers contracted Pontiac Fever (Gregersen et al., 1999; Yanko, 2005). Moreover, Yanko (2005) speculates that the bacteria should grow well in "warm, self-composting organic masses." However, there is no known case of Legionnaires' Disease associated with the production or land application of biosolids.

2.2.1.9. *Screening Bacterial Pathogens*

Some bacteria may be excluded from consideration in risk assessments of pathogens in biosolids. Experts believe that *Staphylococcus aureus* "are not a likely

source of…human exposure or infection" (Pepper et al., 2006). In a study of 23 biosolids samples (16 Class B samples) from 15 U.S. sites, none contained *S. aureus* (Rusin et al., 2003a). Similarly, analyses of 37 air samples were also negative for the bacterium (Rusin et al., 2003a). Although there is little information on the fate of *Vibrio cholera* in biosolids treatment or land application, Yanko (2005) recommends that the low incidence of this disease in the U.S. (0-5 cases per year) is a good justification for focusing research on other pathogens.

2.2.1.10. *Ranking Bacterial Pathogens*

Risk assessors may prioritize bacterial pathogens for inclusion in their risk assessments of land application of biosolids. A workgroup of biosolids experts developed methods for evaluating 20 potential pathogens in biosolids (Chapter 4 in [Smith et al., 2005]). They considered their public health significance (number of infections or severity of disease), prevalence in biosolids and sewage sludge, survival during wastewater treatment and the availability of appropriate analytical methods. Similar criteria might be used by risk assessors in the problem formulation.

2.2.2. Viruses

Over 140 types of enteric viruses are excreted by humans and may be present in municipal wastewater and possibly biosolids (Gerba et al., 2002).

2.2.2.1. *Enteroviruses*

The enteric viruses most often detected in polluted waters are the enteroviruses, though this may be an artifact of the ease of detection in animal cell culture (Pepper et

al., 2006). These include poliovirus, Coxsackie virus, echovirus and enteroviruses 69-91. Both fecal-oral and respiratory routes of infection are common. Enteroviruses are commonly isolated from untreated biosolids. Generally, they are reduced by 90% or more during Class B processes such as aerobic and anaerobic sludge digestion (Pepper et al., 2006).

2.2.2.2. *Rotaviruses*

These are the only double-stranded RNA viruses transmitted through water to humans (NRC, 2002). Along with caliciviruses, rotaviruses are the leading cause of gastroenteritis in the U.S. (Monroe et al., 2000) and a major cause of hospitalization of children in the U.S. (Gerba et al., 1996). These viruses cause waterborne and foodborne outbreaks in the U.S. They have been detected in wastewater, but little information is available regarding their occurrence in biosolids (NRC, 2002).

2.2.2.3. *Caliciviruses*

Caliciviruses may be the leading cause of water and foodborne illness in the world and are a leading cause of viral gastroenteritis (Monroe et al., 2000). The two genera are the Norwalk viruses and the Sapporo viruses (NRC, 2002). Little is known about their environmental occurrence and fate because caliciviruses have not yet been grown in cell culture (Gerba et al., 2002; NRC, 2002).

2.2.2.4. *Adenoviruses*

These common and persistent viruses in wastewater (NRC, 2002) are the second most common cause of childhood viral diarrhea (Gerba et al., 1996). The

mortality of immunocompromised people (e.g., organ transplant, cancer chemotherapy patients) ranges from 53%-69% (Gerba et al., 1996). NRC (2002) provides references indicating that recreational and drinking waters are pathways of exposure for adenoviruses. Adenoviruses are present in untreated sewage sludge (Gerba et al., 2002). Enteric adenoviruses have been detected in Class B biosolids (Sabalos, 1998; NRC, 2002), and adenovirus type 40 has been detected in anaerobically digested biosolids (NRC, 2002). Along with hepatitis A virus, adenovirus is the most thermally resistant virus (Gerba et al., 2002). Little more is known about removal by Class B treatment processes (Gerba et al., 2002).

2.2.2.5. *Astroviruses*

These viruses are a cause of gastroenteritis, primarily in children. Foodborne and waterborne outbreaks have occurred in the past. They have been found in biosolids (Chapron et al., 2000), though still little is known about their removal by Class B treatment processes (Gerba et al., 2002).

2.2.2.6. *Hepatitis A*

This picornavirus is responsible for infectious hepatitis, is transmitted by food and water and primarily infects the liver. The highest infection rate is among children 5 to 14 years old (CDC, 1999). Along with adenoviruses, Hepatitis A is the most thermally resistant virus (Gerba et al., 2002). No information is available on the prevalence of Hepatitis A in biosolids.

2.2.2.7. *Hepatitis E*

This picornavirus, transmitted by the fecal-oral route, has been responsible for major waterborne disease outbreaks in developing countries but has also been reported in frequent travelers to those regions. It is the major cause of acute viral hepatitis in developing countries (Gerba, 2005). Symptoms include jaundice, fatigue, abdominal pain and nausea. Hepatitis E is a more serious infection than Hepatitis A, with case fatalities of 2 to 3% in the general population and 20 to 30% in pregnant women (Haas et al., 1999). No information is available on the prevalence of Hepatitis E in biosolids.

2.2.2.8. *Screening Viral Pathogens from Consideration*

Some viruses may be excluded from consideration by risk assessors of pathogens in biosolids. A workgroup on viruses in biosolids concluded that blood-borne viruses such as HIV would be likely to be inactivated during wastewater or biosolids treatment (Smith et al., 2005b). This workgroup also concluded that lipid-containing viruses have low viability in water and may not survive wastewater or biosolids treatment. However, they recommended that lipid-containing viruses such as rhinoviruses, influenza viruses and herpes viruses not be excluded from consideration until it is known whether any survive treatment (Smith et al., 2005b).

2.2.3. Protozoa

Cryptosporidium and *Giardia* are the predominant protozoan parasites transmitted through food and water in the U.S. that cause diarrhea. These parasites of the small intestine have environmentally resistant stages called cysts or oocysts. Pepper et al. (2006) review studies in which *Cryptosporidium* and *Giardia* have been

detected in sewage sludge and biosolids. Oocysts do not survive under low moisture or high heat conditions, and therefore would be expected to be inactivated during treatment and land application. This expectation has been confirmed by Bowman et al. (2000), who found that these protozoa died within days of Class B biosolids treatment. However Pepper et al. (2006) suggest that new cell culture methods are needed to assess protozoan oocyst viability and confirm that these organisms do not present a hazard in biosolids.

Additional protozoa could be present in sewage sludge and/or biosolids (Bowman and Fayer, 2005). *Cyclospora* causes diarrhea, nausea, vomiting and abdominal cramps. *Toxoplasma gondii* causes neurologic flu-like symptoms, retinitis and severe disfunction in fetuses if mothers are infected for the first time while pregnant. Microsporidia cause diarrhea. *Entamoeba histolytica* causes severe dysentery and extra-intestinal abscesses. *Balantidium coli* causes diarrhea and constipation, but Bowman and Fayer (2005) suggest that their presence is less likely in biosolids than that of other protozoa. Life histories of all of these species, as well as potential effects of biosolids treatment, are summarized in Bowman and Fayer (2005).

Bowman and Fayer (2005) consider the potential hazards of various protozoa by summarizing information on settling rates in wastewater and considering potential resistance to disinfection. “Soft-shelled” protozoa (*Balantidium*, *Entamoeba* and *Giardia*) will probably persist in effluents but not in biosolids. The Apicomplexan protozoa (*Cryptosporidium*, *Cyclospora*, *Toxoplasma*) probably react similarly (but sometimes uncertainly) to the effects of different disinfection methods but settle at

different rates. Microsporidia have not been studied much in the context of biosolids treatment (Bowman and Fayer, 2005).

2.2.3.1. *Helminths*

Several helminth species potentially occur in biosolids. Eggs of many helminth species probably settle in wastewater, are resistant to sewage treatment methods, and end up in biosolids (Bowman and Fayer, 2005).

2.2.3.2. *Trichuris trichuria*

Trichuris (whipworm) is a genus of nematode that is parasitic in the cecum and large intestine of mammals. It causes diarrhea. Human infections result from ingestion of infected eggs. Eggs in wastewater would be expected to settle rapidly and be found in sewage sludge wherever infected people are present in the community (Bowman and Fayer, 2005). Eggs are not likely to be damaged by usual quantities of ultraviolet, ozone, or chlorination disinfection methods.

2.2.3.3. *Ascaris lumbricoides*

Ascaris is a genus of nematode that is parasitic in the small intestine. Adult worms may develop within the small intestine and cause digestive disturbances. Transitory liver and lung disease is caused by larval migration (Bowman and Fayer, 2005). Human infections with *Ascaris lumbricoides* result from ingestion of infected eggs. The eggs of *Ascaris* were chosen as an indicator organism in biosolids because of their resistance to most treatment processes and representativeness of helminth egg viability.

2.2.3.4. *Taeniid Tapeworm Eggs*

The life histories of taeniid tapeworms require a carnivore final host in which the small intestine is infected (Bowman and Fayer, 2005). For *Taenia solium* and *Taenia saginata*, the final host is a human or pig, and the intermediate host is a pig or cow, respectively. The adults cause little effect in humans, but eggs can cause enteric distress. Although *Taenia* species are usually acquired from ingestion of beef, the eggs of this pathogen have been detected in some biosolids (Barbier et al., 1990).

2.2.4. Endotoxins

Endotoxins are nonspecific lipopolysaccharide-protein complexes created from the cell walls of gram-negative bacteria (DeLuzio and Friedman, 1973). They consist of polysaccharide chains connected by a core oligosaccharide to a lipid portion, consisting of a series of long-chain fatty acids, connected by amide and ester linkages to a phosphorolated diglucosamine structure (Epstein, 2006). They may become airborne when dried, pulverized to micron and submicron size particles, and agitated (Smith et al., 2005a). In the bloodstream these toxins may cause a broad range of physiological effects, including fever, coughing, breathlessness, flu-like symptoms, inflammation and shock (Yanko, 2005; Pepper et al., 2006; Epstein and Moss, 2006). Endotoxins are relatively heat stable (Epstein, 2006).

Endotoxins have been measured in air at composting plants, though there was no evidence of residential impact because levels decreased to background concentrations beyond site boundaries (Clark et al., 1983; Pepper et al., 2006). Ambient levels of dust-associated endotoxin are high (Smith et al., 2005a; Pepper et al., 2006). Endotoxin levels in Class B biosolids are similar to concentrations in animal

manures and composts (Brooks et al., 2006). Farming activities, such as driving a tractor across a field, result in comparable levels of aerosolized endotoxins as those from land application of biosolids (Brooks et al., 2004). Low concentrations of endotoxins were present in groundwater at two sites where wastewater was applied to land (Yanko, 2005).

2.2.5. Emerging Pathogens

The lists of pathogens covered in this document should not be considered exhaustive. New pathogens are continually being identified or found in new areas for several reasons: changes in the way foods are produced, the global transportation of food and people, advances in molecular biology that permit the identification of new pathogens and their sources, the evolution of pathogens, aging demographics and the use of microbial risk assessment to quantify risks from environmentally transmitted pathogens (Gerba and Smith, 2005). Emerging pathogens are novel pathogens that have not previously been characterized or established pathogens that have only recently been considered stressors of concern in particular media. Gerba et al. (2002) designated *E. coli* O157:H7, *H. pylori* and *L. montogenes* as newly emerging bacterial pathogens of potential concern in biosolids. Yanko (2005) points out that many of these emerging bacterial pathogens do not fit the classic fecal-oral transmission pattern. The NRC listed *Mycobacterium*, *E. coli* O157:H7, *Legionella*, *Listeria* and Microsporidia as emerging bacterial pathogens likely to be present in biosolids and Adenovirus, Norwalk virus, Astrovirus, Hepatitis A, Rotavirus and Hepatitis E as emerging viral pathogens likely to be present (NRC, 2002). Gerba (2005) listed several emerging viruses without

speculating which are likely to be in biosolids: picobirnaviruses, picotrinaviruses, coronaviruses and toroviruses.

NRC (2002) identified criteria for selecting emerging pathogens for which additional information on occurrence, persistence, and risk is justified, and for which additional regulations may be needed. These criteria, suggested by C. Gerba of the University of Arizona, are useful for selecting pathogens on which to focus the stressor characterization in a risk assessment.

- Reliable viability assay
- Wastewater-related disease-causing agents
- Extent of existing data on probability of surviving biosolids treatments (organisms surviving at high pH above 11-12 and heat resistance are of greatest concern)
- Extent of survival in the environment

Based on these criteria, NRC (2002) recommended *E. coli* O157:H7, adenovirus 40, astrovirus, hepatitis A virus and rotavirus in biosolids as priorities for analysis. The committee would have selected caliciviruses as a priority, but methods of assessing viability are not available (NRC, 2002). Similarly, *Legionella* merits investigation, but current detection methods are inefficient, difficult to use and expensive (NRC, 2002).

2.2.6. Multiple Stressors

It may be reasonable to assume that microbial pathogens act independently of each other and that the probability of an adverse effect from one pathogen is independent of the probability of an adverse effect from another. However, assessors

of cumulative risks should consider exposures to offsite pathogens in biosolids or other sources that are not the direct subject of a biosolids risk assessment.

There is no evidence to suggest that pathogens and chemicals such as metals in biosolids have interactive effects in humans. However, Lewis et al. (2002) speculated that chemical contaminants in biosolids might irritate the skin and mucous membranes and thus increase pathogen host susceptibility.

3. DEVELOPMENT OF CONCEPTUAL MODELS, ENDPOINTS AND SCENARIOS

A conceptual model for a risk assessment is a representation of the assumed relationships between sources and effects (Suter, 1999) or between stressors and assessment endpoints (U.S. EPA, 1998). Multiple models may be developed for multiple scenarios. The written descriptions of the risk hypotheses, accompanied by diagrams (termed conceptual models) that illustrate the key relationships, are among the primary products of the problem formulation (U.S. EPA, 1998). Conceptual models "provide a framework for prediction and are the template for generating more risk hypotheses." They form the basis for developing quantitative exposure and effects models for the risk assessment. The models tend to emphasize exposure pathways, including indirect exposures, over mechanisms of effects. Conceptual models are much more common in ecological risk assessment than in human health risk assessment, and conceptual models for human health risk assessments of pathogens in biosolids that include detailed source descriptions, transport pathways and routes of exposure have not been developed previously.

In this report we develop conceptual models illustrating the potentially important human exposure pathways for pathogens in biosolids that have been applied to land. These models are developed in response to NRC's assertion that "EPA should develop a conceptual site model to identify the major and minor exposure pathways (including secondary transmission) by which humans might come into contact with pathogens in biosolids" (NRC, 2002). The models are applicable to biosolids amendments to cropland, pasture land, forests, mineland (for reclamation), or other uses. The conceptual models presented here are limited to primary transmission, i.e., exposure of

humans to pathogens from biosolids without an intermediate human host. Secondary transmission is infection by pathogens that were shed by infected people. This problem formulation does not provide advice concerning estimates of secondary infection because the process is not unique to pathogens in biosolids. This does not mean that secondary transmission of pathogens in this context is assumed to be unimportant.

Some of the primary differences between conceptual models for pathogen risk assessments and conceptual models for chemical risk assessments are that: (a) some microorganisms can reproduce in the environment, (b) host factors such as individual immunity and genetic factors influence disease and (c) infection may occur via person-to-person transmission (Soller et al., 2006), though that transmission pathway is not treated here.

The conceptual models presented in this report are not meant to imply that the risk assessor must assume that adverse health effects are caused by exposure to pathogens in biosolids. A causal association between exposures to biosolids and adverse effects on human health has not been documented.

In this chapter we first present a general conceptual model for risks from pathogens in land-applied biosolids (Figure 1), as well as a narrative description of the model. The model is a cascade of processes and states (Suter, 1999) that indicates the mechanisms by which the pathogen stressors potentially contact human hosts to produce infection and disease. We describe the source (methods and rates of land application), environmental fate and transport processes, routes of exposure, host susceptibility factors, infection and disease. Then we describe five exposure scenarios, along with related generic conceptual models, that are of interest

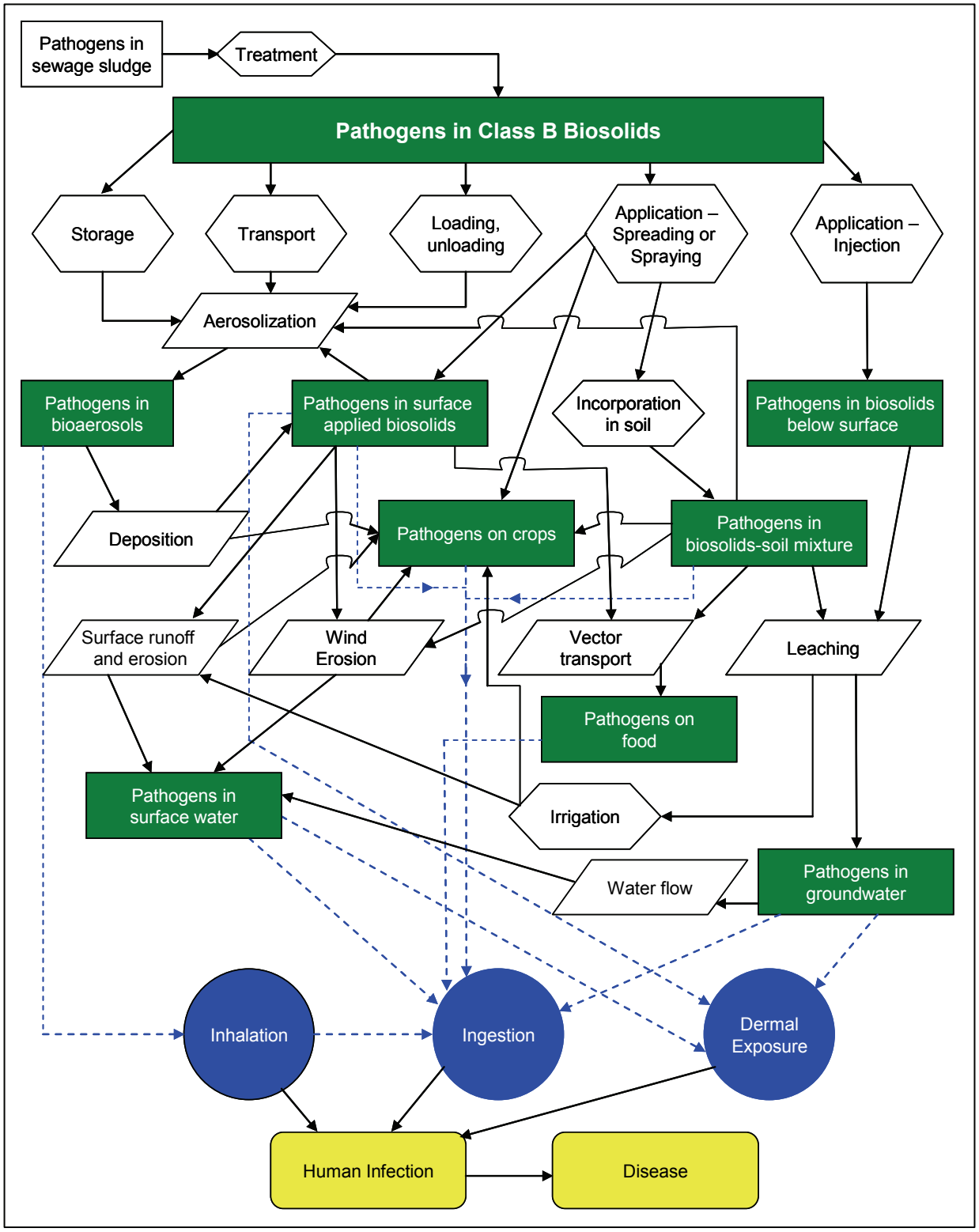

FIGURE 1

General Conceptual Model

for assessing risks from the land application of biosolids. The generic conceptual models presented here may be modified as more knowledge is available on a case-by-case basis.

The model contains routes of exposure that are considered to be potentially significant in many instances. However, some additional routes may be considered when there is a particular concern. For example, indirect routes involving human consumption of livestock, dairy products, wildlife, fish or shell fish that are exposed to pathogens from biosolids were not included as too indirect and hypothetical. However, such routes should be considered if they are an important issue for stakeholders at a site.

Site-specific conceptual models that make use of these generic models would be needed for site-specific risk assessments. Site-specific conceptual models can be generated from these generic models by eliminating routes that are impossible or highly improbable at the site, adding routes that are peculiar to the site and adding details. In the next chapter, we screen out pathways that usually contribute negligible human exposures to biosolids-derived pathogens.

3.1. PREAPPLICATION PROCESSES

Various treatment processes are not separate boxes in the conceptual model because all treatment technologies are assumed to be operating as intended, generating Class B biosolids (Figure 1). Additional human processes in the conceptual model include storage, transport within a site, loading and unloading and land application (Figure 1).

Biosolids storage, transport within a site and loading and unloading processes are included in the general conceptual model because these processes have been observed to generate bioaerosols ([Pillai, 2007; Paez-Rubio et al., 2007], Figure 1). Biosolids are stored during winter, inclement weather, periods of equipment breakdown, or crop growth periods (Evanylo, 1999). Regulations may specify the type of storage facility for long-term storage, and this problem formulation assumes that a barrier is present to prevent erosion of biosolids or surface runoff or leaching of pathogens. Thus, there is no arrow between storage and surface runoff and erosion or leaching in Figure 1. However, if risk assessors determine that leaks of biosolids or pathogens from storage facilities are feasible, then additional pathways from the storage facility must be included in the conceptual model. Dewatered biosolids are stockpiled, and liquid biosolids may be stored in digesters, tanks, lagoons or drying beds (Evanylo, 1999).

3.2. APPLICATION

3.2.1. Methods of Land Application of Biosolids

The three major methods of biosolids application are injection, surface application without incorporation into soil, and surface application with incorporation into soil. Methods depend on the water content of biosolids, land use, site topography, quantity of debris, presence of obstructions such as trees, presence of waterways, climate and the availability of application equipment (NRC, 2002; University of Washington, 2002), and state or local regulations (e.g., Solano County, California requires incorporation of biosolids into soil). The application method is an important

determinant of bioaerosol generation, chemical odor and ultraviolet inactivation of pathogens (NRC, 2002).

Subsurface injection of liquid biosolids involves small-diameter injection tubes to minimize soil disturbance or disking if soil turnover is desired in farm management practices (NRC, 2002). Injection is typically at a depth of 6 to 9 inches (15-23 cm) and usually occurs before planting or after harvest (NRC, 2002). Injection reduces odor and risk of runoff to surface water (NRC, 2002) as well as preventing aerosolization of biosolids (Figure 1). As would be expected, Gerba et al. (2002) found that injected biosolids presented a much lower risk of infection from ingestion than surface-applied biosolids without incorporation. Hence, injection is treated separately from surface application in the conceptual model (Figure 1). Injection can be used on slopes up to 15 percent (Evanylo, 1999), dependent on state or local laws. This application method serves as a physical barrier that satisfies vector-control requirements (NRC, 2002). Injection or soil incorporation is rarely used for pasture or hay crops. Application under any circumstance is prohibited for any land use when the ground is frozen (NRC, 2002; U.S. EPA, 1993).

Surface application involves the application of liquid biosolids or cake solids to the soil surface. Liquid biosolids are typically pumped and sprayed through a cannon or spray nozzle. Solid biosolids are flung from a manure-type spreader or dumped from a truck. Where application is to a forest, a portion of the sprayed biosolids may coat tree surfaces prior to washing down to soil surfaces. In some climates and at high depths of biosolids, drying of the material may require a complete summer period. Drying can be promoted by seeding with a grass such as annual rye or wheat that can germinate and

survive in fairly anaerobic conditions (University of Washington, 2002). In contrast to injection, surface application is commonly used for hay crops and winter applications. Stabilization of biosolids to meet vector-control requirements must occur through treatment prior to surface application. Surface application permits ultraviolet inactivation of viruses (NRC, 2002). Spreading of dewatered biosolids may sometimes produce higher bioaerosol emission rates than spraying of liquid biosolids (Paez-Rubio et al., 2007).

Incorporation of cake biosolids into soil through plowing or disking at a depth of 6 to 9 inches (15 to 23 cm) may follow surface application (NRC, 2002) and partial drying (Evanylo, 1999). The method is usually used before planting or after harvest (NRC, 2002). Surface application with incorporation is generally limited to soils with less than a 7 percent slope (Evanylo, 1999), additional state and local laws notwithstanding. Incorporation serves as a physical barrier that satisfies vector-control requirements (NRC, 2002).

Application methods vary with region and type of biosolids. In the arid and semiarid southwest, liquid anaerobic-digested biosolids are typically injected into the soil subsurface (NRC, 2002). On pasture land, the material tends to be applied to the soil surface, as incorporation is more difficult than on crop land (NRC, 2002). Similarly, incorporation is not common in forests. In many agricultural lands, biosolids cakes are disked into soil (NRC, 2002).

3.2.2. Rates of Land Application of Biosolids

Biosolids are applied at a rate equal to or less than the agronomic rate (nitrogen needed by crops, trees, or other vegetation). Rates of application are generally calculated on a dry weight basis. Information on application rates from the 1980s is summarized in Table 3. Notably, the rate of application at reclamation sites is usually much higher than that at farm sites (NRC, 2002). However, agricultural sites are more likely to involve multiple applications (NRC, 2002). U.S. EPA has predicted that cumulative pollutant loading limits for the application rates in Table 3 will be reached after 100 years for agriculture, 55 years for forest, 32 years for public contact, and 13 years for reclamation, assuming annual applications (NRC, 2002; U.S. EPA, 1992). Applications are assumed to cease when cumulative loading limits are reached.

TABLE 3

Estimated Biosolids Application Rates for Different Land Uses

Land Use	No. Observations	Mean Application Rate (metric tons/ha/yr of dry wt)	Standard Deviation	75^{th} Percentile (metric tons/ha/yr of dry wt)
Agriculture	87	6.8	105	16
Forest	2	26	26	34
Public contact	11	19	122	125
Reclamation	7	74	148	101

Sources: NRC (2002) and U.S. EPA (1992).

3.2.3. Timing of Land Application of Biosolids

The timing of land application of biosolids is another factor that determines exposure. In agricultural operations, application is scheduled around tillage, planting and harvesting and is also influenced by properties of crops, climate and soil factors (Evanylo, 1999). Most applications are performed when plants are ready to use the nitrogen in biosolids so as to minimize leaching to groundwater (Evanylo, 1999). The State of Virginia recommends that biosolids applied to land between fall and spring have a vegetation cover to minimize runoff of pathogens and nutrients and erosion of sediment-bound biosolids (Evanylo, 1999). However, spray irrigation is not recommended for applying biosolids to forage, row crops, or young tree stands during the growing season, because adherence to leaves can reduce photosynthesis (Evanylo, 1999; McFarland, 2000). Workers who apply biosolids avoid periods of rain, because vehicles may compact or create ruts in soils that reduce crop yields (Evanylo, 1999).

Although rain is avoided during application of biosolids, we have found no evidence that heavy winds are similarly avoided. Meteorology should be considered in the modeling of transport of biosolids.

3.2.4. Regional Application Issues

Exposure factors that vary by region include methods of biosolids application, climate, soils and land available for application in relation to human populations. A few regional differences in application methods and timing are described above. Climatic differences contribute to differences in fate and transport of pathogens in biosolids and biosolids-amended soil. Pathogen survival tends to be highest in cool, moist soils, such

as those in the northeastern U.S. Hot, dry soils as in the southwestern U.S. contribute to pathogen mortality (see section below on fate and transport of pathogens). Differences in rainfall are counteracted by irrigation in drier climates. Groundwater contamination by pathogens from biosolids is most likely in coarse-textured, sandy soil or land underlain by high permeability karst (NRC, 2002).

The number of people potentially affected by pathogens in biosolids also varies regionally. Potential exposure increases as the density of people increases because (1) greater sewage sludge output leads to a greater need to find land application sites and to apply biosolids at higher rates and (2) the greater density of people means more residents and children potentially exposed near their homes and schools. In the arid southwestern U.S., farms are often located far from cities, so fewer residents would be expected to be exposed to pathogens in biosolids (NRC, 2002).

3.3. FATE AND TRANSPORT OF PATHOGENS

3.3.1. Pathogen Survival, Growth and Death

As stated in the stressor characterization chapter, unlike chemical stressors, biological stressors have the potential to reproduce or to die. Thus, conceptual models need to consider factors affecting survival and growth in biosolids, biosolids-amended soils and bioaerosols (Figure 2). The environmental factors affecting survival of viruses, bacteria and protozoa are presented in Table 4 (Bujoczek et al., 2001; Gerba et al., 2002; Pepper et al., 2006; NRC, 2002). Most enteric pathogenic bacteria are non-spore-formers and relatively sensitive to environmental factors such temperature, desiccation and ultraviolet exposure. Although *Salmonella*, *E. coli* and fecal coliforms are capable of regrowth in moist conditions following treatment, regrowth is typically

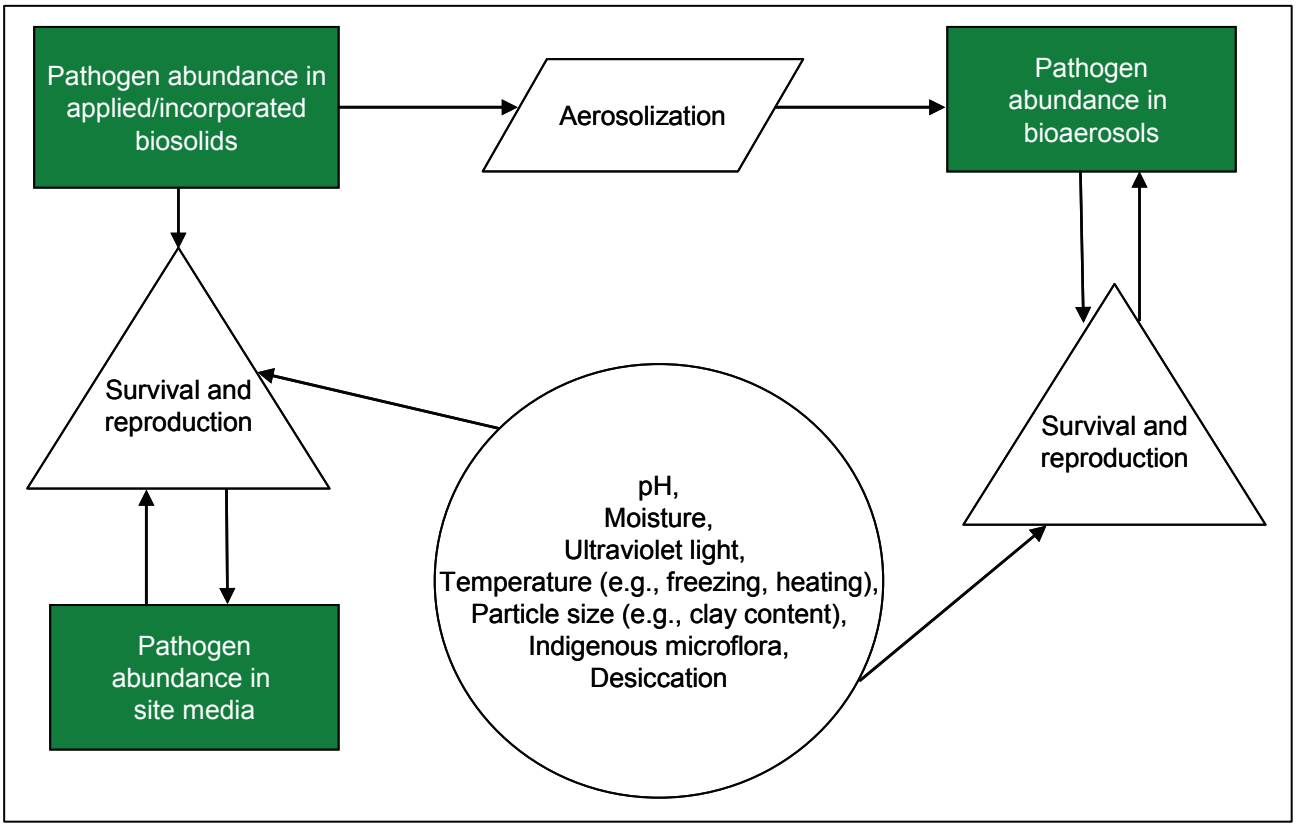

FIGURE 2

Pathogen Fate Conceptual Model

1

TABLE 4

Environmental Factors Positively or Negatively Affecting the Survival of Pathogenic Microbes

Parameter	Survival time		
	Virus	Bacteria	Protozoa
Temperature increasing	–	–	–
Soil moisture decreasing	–	–	–
Rate of dessication increasing	–	–	–
Clay content increasing	+	+	Not known
pH range of 6-8	+	+	+

2

3 Sources: NRC (2002) Pepper et al. (2006).

limited to Class A biosolids where biological competition is low compared to Class B biosolids (Pepper et al., 2006).

Pathogen survival and reproduction are depicted in Figure 2. Temperature and moisture are the primary variables affecting survival of enteric viruses in soil (Gerba et al., 2002). In addition to the mechanisms in Table 4, ultraviolet light has the potential to attenuate pathogens, especially those that have been aerosolized (Paez-Rubio and Peccia, 2005; Pepper et al., 2006). Viruses vary considerably in their ability to survive outside a host organism. *Ascaris* eggs may survive several years in soils that are not very wet or very dry (NRC, 2002). Little is known about the viability of protozoa following land application of biosolids (NRC, 2002). Even less is known about the survival and reproduction of pathogens in bioaerosols than about their survival in biosolids or biosolids-amended soil.

3.3.2. Pathogen Transport

Pathogens may be transported from biosolids to various media. In addition to the application process, storage, site-to-site transportation and loading and unloading are human processes that could mobilize pathogens for transport (Figure 1). Several mechanisms of transport are possible: aerosolization followed by aerial transport and deposition, erosion, surface runoff and leaching to groundwater (Figure 1).

3.3.2.1. *Aerial Transport*

Land application of biosolids can generate bioaerosols either through agitation during application or following a series of weathering events of deposited biosolids in association with specific climatic conditions (see stressor characterization). Biosolids

left on the soil surface or lightly incorporated may be subjected to conditions that lead to drying of the material, rendering it friable. Particulates generated from the friable material are capable of becoming airborne along with the associated pathogens. Bioaerosol droplets or particles are generated at the site of biosolids application, storage, site-to-site transport and loading and unloading processes, including shoveling biosolids from pile to pile (Straub et al., 1993; Pillai, 2007, Figure 1). Bioaerosols are potentially transported to downwind locations. Wind can resuspend biosolids that have been previously applied to the soil surface through the wind erosion process in Figure 1. Injection is a barrier to aerosolization of biosolids (Smith et al., 2005a, Figure 1).

The disking process, marked as “incorporation in soil” on Figure 1, can be a “substantial source of biosolids-derived aerosols” (Paez-Rubio et al., 2006). The emission rate of pathogens during disking of biosolids may be greater than rates during spreading of dewatered biosolids by side slinger or spraying of liquid biosolids (Paez-Rubio et al., 2006). Aerosol emission rates from dewatered biosolids may be higher than those for liquid biosolids (Paez-Rubio et al., 2007). In one study, loading and unloading operations were responsible for the highest predicted annual risks of infection by coxsackievirus A21 at a distance of 30.5 m (Brooks et al., 2005b).

The launch patterns of bioaerosols from localized sources of biosolids have a conical dispersion form, whereas bioaerosols originating from more spatially extensive fields have a particulate-wave type of dispersion (NRC, 2002). Both the application and incorporation processes, as well as site-to-site transport provide moving sources of aerosols. In addition to the source, the physical properties of aerosols and environmental settings affect the dispersal and settling of bioaerosols. Physical

properties include the size, density and shape of droplets or particles. Precipitation, relative humidity, temperature and air currents can affect dispersal and deposition of aerosolized biosolids (Pillai, 2007).

Evidence from Tanner et al. (2005) suggests that under some conditions, aerosolized viruses may be transported farther than aerosolized gram-negative bacteria.

3.3.2.2. *Runoff to Surface Water*

Water-borne exposure to pathogens from biosolids is driven by precipitation sufficient to move the organisms from the site of application to surface water as runoff (NRC, 2002). The movement of pathogens associated with applied biosolids to surface water depends on the numerous environmental properties of the area where the biosolids were applied as well as those of adjacent lands. Runoff of pathogens to surface water is expected to be higher where the biosolids are left on the surface (e.g., in forests) compared with incorporation into cropped soils. The NRC noted that U.S. EPA did not adequately consider the potential for contamination of neighboring properties or surface water by runoff in the Part 503 rule (NRC, 2002). Smith et al. (2005b) identified the monitoring of pathogens in runoff from land application of biosolids to be a research priority, because little is known about this transport pathway.

3.3.2.3. *Erosion to Surface Water*

Where biosolids are applied to the soil surface, runoff may transport particles to surface waters down-gradient (Straub et al., 1993), at least “in principle” (NRC, 2002). Disking operations also break up and mix the biosolids with soil, which increases the

potential for erosion and runoff but buries the amendment and dilutes the initial numbers of pathogens. However, we have found no studies of microbial contamination of surface water where biosolids have been applied.

3.3.2.4. *Leaching to Groundwater*

Following precipitation, microorganisms may infiltrate soil to contaminate groundwater (Straub et al., 1993). The NRC noted that U.S. EPA did not adequately consider the potential for contamination of groundwater by runoff in the Part 503 rule (NRC, 2002). The transport of microorganisms through soils is affected by both abiotic and biotic factors, including adhesion processes, filtration effects, physiological state of the cells, soil characteristics, water flow rates, predation, intrinsic cell mobility and presence of biosolids (NRC, 2002). Viruses have a greater potential to be transported to groundwater than other pathogens, though sorption to colloids and biosolids particles limits this potential (NRC, 2002). Transport of larger organisms (bacteria, protozoa, helminths) is less likely but possible if preferential flow occurs through cracks and macropores (NRC, 2002). Transport of pathogens to groundwater is most likely where soils are sandy and coarse-textured or where karst topography is present (NRC, 2002). However, we have found no studies of microbial contamination of groundwater where biosolids have been applied.

3.3.2.5. *Sorption to Crops*

Pathogens from biosolids could become sorbed to root crops with particles from the biosolids-soil mixture (Figure 1). Although crops are generally washed before eating, a fraction of biosolids-amended soil will remain sorbed to the crop (estimated at

10% by Gale [2005b]). This pathway is likely the dominant route to crops. Additional pathogens might become sorbed to root crops following runoff from biosolids-amended fields to neighboring fields. Leaf crops might become contaminated with pathogens deposited from bioaerosols or rainsplash (Figure 1). Leaf or root crops could become contaminated with pathogens via irrigation with contaminated surface water or groundwater (Figure 1).

3.3.3. Vector Transport

Vector transport of pathogens from biosolids is possible. For example, flies might become contaminated, leaving trace pathogens on food that is ingested by humans. This potential pathway is included in the general conceptual model (Figure 1). No information is available on the extent to which land application of biosolids attracts flies or other potential vectors, such as mosquitoes or birds (NRC, 2002). Pets are a potential vector, resulting in dermal, oral (hand to mouth) or respiratory exposures. It is unclear whether procedures in the Part 503 rule that are intended to discourage vectors are effective (NRC, 2002). Similarly, it is unclear whether vectors are involved in the transmission of pathogens to humans from biosolids (NRC, 2002).

3.4. HUMAN ROUTES OF EXPOSURE

Potential routes of exposure to pathogens originating in biosolids include ingestion, inhalation and dermal exposure (Figure 1). Whereas all of these routes are feasible, none has been implicated in disease. Risk assessors should consider all of these potential routes, unless fewer routes are specified in a scenario of interest.

3.4.1. Inhalation

The route of exposure of humans to aerosolized pathogens is uncertain, involving a combination of inhalation and ingestion (Pillai, 2007, Figure 1). Large aerosolized particles (between 5 and 20 μm) can deposit in the upper respiratory tract. Clearance of these particles results in oral exposures. Smaller particles penetrate deep into the lungs, with many retained by the alveoli (Pillai, 2007). Thus, inhalation is the most probable route of exposure to smaller particles. In one study that investigated bioaerosols emitted during the spreading of dewatered Class B biosolids onto farm land, the diameters of most emitted particles were of inhalable and possibly respirable size (Paez-Rubio et al., 2007). Because of the high volume of air that is inhaled daily, Pillai and Ricke (2002) assert that inhalation is the predominant route of exposure to aerosolized pathogens that may result in adverse health effects.

The NRC (2002) determined that the inhalation pathway was among the routes of exposure that was not adequately assessed by U.S. EPA in the development of the Part 503 rule. They noted that inhalation of dust was presumed by U.S. EPA to occur only on-site and that controlling site access was thought to prevent that route of exposure (NRC, 2002). We did not locate studies of inhalation of biosolids-derived aerosols or pathogens by off-site residents. Thus, inhalation of pathogens by off-site residents needs more consideration.

3.4.2. Ingestion

Ingestion of biosolids-related pathogens may occur via several exposure scenarios including; direct and incidental ingestion of surface or groundwater containing

pathogens that originated in biosolids; ingestion of pathogens which are sorbed to crops and food items after application of biosolids in agricultural fields; incidental ingestion pathogens associated with surface-applied biosolids and biosolids mixed with soil, and ingestion of bioaerosols containing pathogens (Figure 1).

Ingestion of biosolids in soil occurs through the transfer of pathogens to the mouth from contaminated hands or crops and or though inhalation followed by swallowing (Gerba et al., 2002, Figure 1). Larger particles in contact with the respiratory tract can be cleared from the tract and swallowed. Researchers vary in their estimation of the percentage of inhaled organisms that are ingested (Pillai, 2007).

Ingestion of groundwater or surface water is a potential route of exposure to biosolids-derived pathogens (see scenario descriptions below). Untreated surface water contaminated with pathogens from biosolids might be ingested while swimming, potentially allowing for greater consumption of pathogens than domestic consumption from a tap.

Food consumption is a potential direct route of exposure to pathogens, especially involving ingestion of foods not subjected to cooking or washing. Biosolids are applied to agricultural soil to improve its fertility and to enhance crop yields. The application of biosolids to soil along with consumption of food grown on amended fields provides an avenue of exposure to pathogens through the food chain. Reasonable exposure scenarios involve the adherence of the pathogens to the plant (i.e., roots, leaves), particularly the edible portion of the plant, and consumption by individuals.

Three exposure scenarios may result in ingestion of pathogens associated with biosolids when applied in crop settings. The exposure scenarios differ with respect to

the portion of the plant that is intended for consumption. The first scenario involves the deposition of aerosolized material on the surface of the aboveground portions of the plant (Figure 1). This exposure may arise during biosolids application. In this scenario, biosolids may be applied by spreading or spraying the material onto the soil with the resulting generation of airborne pathogens from the biosolids (Figure 1). Pathogens and biosolids material subsequently land on and adhere to the aboveground portion of the plant that is intended for consumption.

Compliance with current regulations makes pathogen ingestion on crops an unlikely exposure pathway for farm residents (see section on regulatory restrictions, below). Part 503 regulations provide for time restrictions between application to the field and harvesting of plants. However, harvesting of plants in nearby fields where pathogen deposition from the air or runoff may have occurred is not restricted. Additionally, the placement of microorganisms on the aboveground portion of the plant subjects the pathogens to environmental stressors such as UV radiation and desiccation, both of which diminish the viability of the pathogens. Moreover, the types of foods that may be affected by deposition of aerosolized material are grains and some vegetables which normally undergo preparation to reduce pathogen viability prior to consumption. Although this scenario might constitute a minor pathway, it should be considered in the problem formulation.

A second exposure scenario addresses plant consumption in which the palatable portion is aboveground but is expected to come in contact with the soil. This scenario includes some fruits and vegetables such as melons, cucumbers and tomatoes. This scenario allows for extended contact with soil while the plant develops with the

possibility of infection of the plant through a lesion or by adherence to the plant surface. Many of the crops that fall into this category include vegetables that are consumed without prior food preparation other than normal washing, which may not apply to all households. However, as the area of contact is with the soil surface, it is anticipated that the pathogens would be exposed to higher levels of environmental stressors which would reduce the viability of pathogens.

A third scenario applies to crops that have the palatable portion below the soil surface. An example is tubers; crops for which the roots serve as the consumable portion of the plant, such as potatoes, carrots and yams. This scenario poses a concern for several reasons. First, this exposure scenario involves direct contact to pathogens with the greatest potential for long-term survival, i.e., those that are found below the soil surface. Furthermore, because the food portion of the plant develops in close contact with the soil, it has the greatest potential for retaining the pathogens on the plant surface. Finally, some tubers may be ingested with little or no preparation that would remove or inactivate pathogens on the edible plant surface. For example, carrots are usually eaten raw. They may be washed or skinned prior to eating, but the amount of preparation varies considerably.

Part 503 regulations address these exposure scenarios for Class B biosolids through appropriate grazing, harvesting and public access restrictions. Existing regulations establish temporal restrictions on the planting, harvesting and consumption of food grown on land receiving Class B biosolids. Nonetheless the potential remains for consuming food harvested from amended plots. As presented in the section on regulatory restrictions (below), Part 503 regulations require a waiting time of either 20 or

38 months for crops whose harvested portion is below ground; shorter periods for crops where the above-ground portion is harvested. Pathogens capable of surviving over this period of time can adhere to the surface of the harvested portion of the plant, and with inadequate food preparation steps, can be consumed.

3.4.3. Dermal Exposure

Dermal contact constitutes a direct method of transfer of pathogens in biosolids to receptors (Figure 1). Dermal exposure to pathogens would occur primarily through skin abrasions, either through contact with contaminated soil or surface water.

Dermal contact may occur during occupational exposure or during unintended contact with biosolids that have moved from the site of application (e.g., through aerial dispersion or runoff). Workers will most likely come in contact with biosolids during processing, loading and application which can lead to penetration of the pathogens through skin or existing cuts or abrasions. However, this problem formulation is concerned with residents and other community receptors rather than workers (Figure 1).

A possible exposure scenario may occur as the result of recreation during the summer months. For example, swimming in surface waters would permit dermal contact with pathogens, as well as ingestion or inhalation.

To assess dermal exposures, the risk assessor would need information on the amount of material adhering to skin and dose-response values for the pathogens of interest as well as data on the distribution and numbers of pathogens in biosolids and their potential for regrowth.

3.5. REGULATORY RESTRICTIONS

Many site restrictions related to land application of biosolids are intended to reduce exposure to pathogens and chemicals in the material (Table 5). These restrictions affect the credibility of exposure pathways in the conceptual model. Time intervals required prior to site access are summarized in Table 6. Particular states may have regulatory criteria for distances to surface waters or wetlands, slope restrictions, depths to groundwater and bedrock, soil permeability rates, distances to residences, schools, health care facilities or recreation areas, and distances to private or public water-supply wells (NRC, 2002).

3.6. FACTORS THAT AFFECT INFECTION AND DISEASE

Several host and pathogen characteristics affect the probability or intensity of disease (Figure 3).

3.6.1. Human Factors

The three host factors that are discussed in NRC (2002) are concomitant exposures, genetic factors and acquired immunity. Age is an additional determinant of susceptibility.

3.6.1.1. *Concomitant Exposures*

Various stressors such as pathogens, noninfectious organisms, cellular components, irritants and odors may influence individual immunity, other aspects of susceptibility, or the nature or intensity of disease (Figure 3). Synergistic effects might

TABLE 5

Pathways of Exposure and Applicable Use Restrictions for Class B Biosolids

Pathways	Part 503 Required Use Restriction
Handling soil from fields where biosolids have been applied	No public access[a] to application until at least 1 year after Class B biosolids application
Handling soil or food from home gardens where biosolids have been applied	Class B biosolids may not be applied on home gardens
Inhaling dust[b]	No public access to application sites until at least 1 year after Class B biosolids application
Walking through fields where biosolids have been applied[b]	No public access to fields until at least 1 year after Class B biosolids application
Consuming crops from fields on which biosolids have been applied	Site restrictions that prevent the harvesting of crops until environmental attenuation has taken place
Consuming milk or animal products from animals grazing on fields where biosolids have been applied	No animal grazing for 30 days after Class B biosolids have been applied
Ingesting surface water contaminated by runoff from fields where biosolids have been applied	Class B biosolids may not be applied within 10 meters of any waters to prevent runoff from biosolids-amended land
Ingesting inadequately cooked fish from water contaminated by runoff from fields where biosolids have been applied, affecting the surface water	Class B biosolids may not be applied with 10 meters of any waters prevent runoff from biosolids-amended land
Contact with vectors that have been in contact with biosolids	All land-applied biosolids must meet one of the vector-attraction-reduction options

[a]Public-access restrictions do not apply to farm workers. If there is low probability of public exposure to an application site, the public-access restrictions apply for only 30 days. However, application sites that are likely to be accessed by the public, such as ballfields, are subject to 1-year public-access restrictions.

[b]Agricultural land is private property and not considered to have a high potential for public access. Nonetheless, public-access restrictions are applied.

Taken from NRC (2002), which adapted the table from U.S. EPA (1999).

TABLE 6

Minimum Time Interval between Application and Harvest, Grazing or Public Access to Lands Applied with Class B Biosolids

Criteria		Injection	Surface Application	Surface with Incorporation
Harvest	Food crops whose harvested parts may contact biosolids-amended soil	14 months	14 months	14 months
	Food crops whose harvested parts grow in soil	38 months	20 or 38 months*	38 months
	Food, feed and fiber crops	30 days	30 days	30 days
Grazing	Animal grazing	30 days	30 days	30 days
Public Access	High potential for exposure	1 year	1 year	1 year
	Low potential for exposure	30 days	30 days	30 days

*The 20-month interval prior to harvesting applies if the biosolids stay on the surface for 4 months or longer prior to incorporation. The 30-month interval applies if the biosolids stay on the surface for less than 4 months prior to incorporation.

Modified from: NRC (2002) and 40 CFR Part 503.

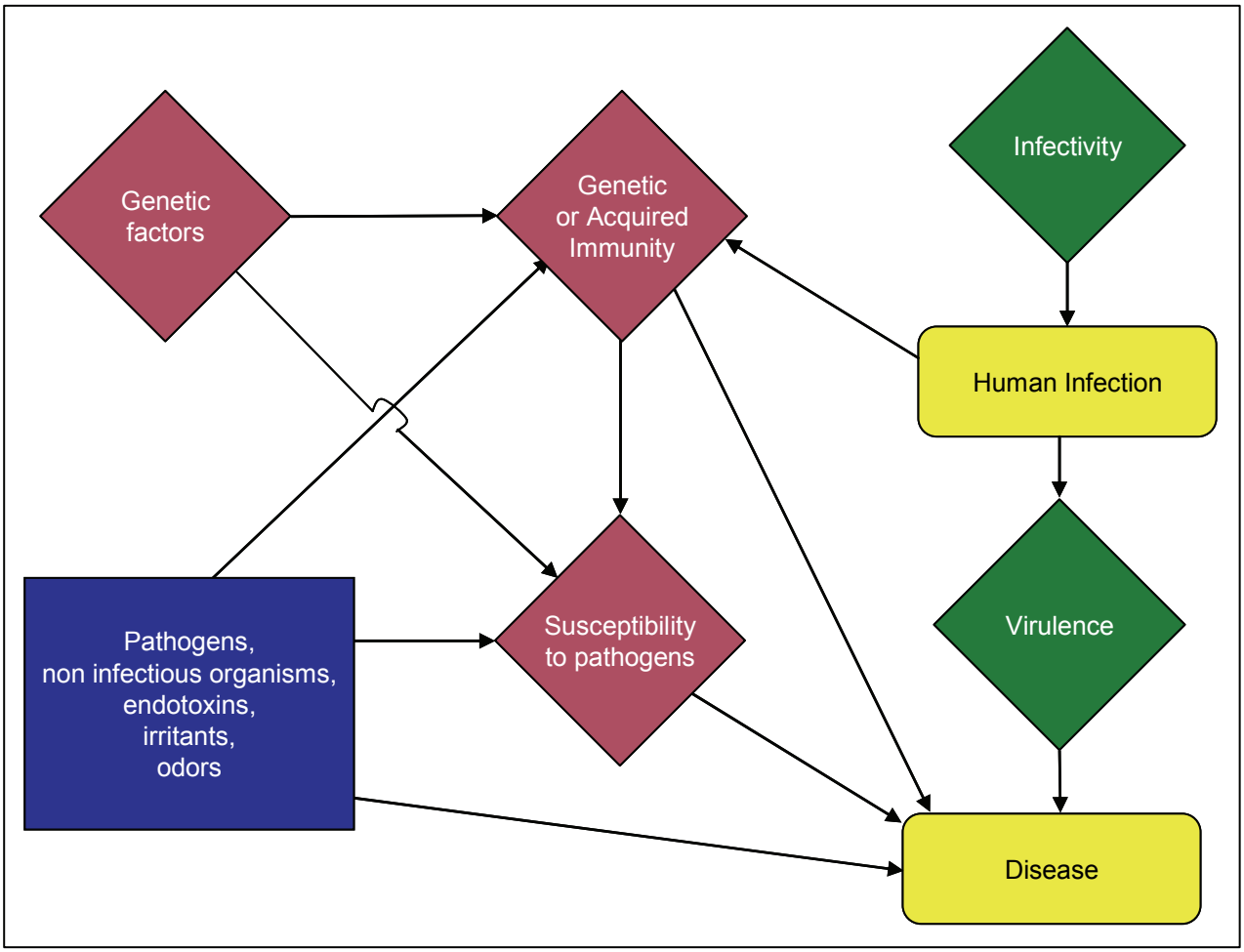

FIGURE 3

Disease Factors Conceptual Model

result from combined exposures to these stressors (NRC, 2002, Figure 3). For example, endotoxins may combine with particles and allergenic components to promote the development of respiratory diseases and systemic effects (NRC, 2002).

3.6.1.2. *Genetic Factors*

Genetic factors influence individual immunity as well as other aspects of disease susceptibility (Figure 3). Genetic factors such as a predisposition to asthma attacks can be a factor in determining whether infection proceeds to disease. No information is available on the role of genetic factors in contributing to health effects due to bioaerosols from land-applied biosolids (NRC, 2002).

3.6.1.3. *Acquired Immunity*

Acquired immunity is the result of previous exposure to pathogens and is part of the immunity box in Figure 3. Acquired immunity can reduce the fraction of illness in a population exposed to pathogens (NRC, 2002). Genetic factors also contribute to the immune status of an individual. The dynamics of immunity are not well understood for most pathogens. Loss of immunity to pathogens is also a possible result of exposure to other pathogens or biological or chemical stressors (Figure 3).

3.6.2. Additional Susceptibility Factors

For public health risk assessment purposes, exposed populations are evaluated based on age (children, adults, geriatrics). In addition, sensitive subpopulations may be evaluated based on gender, ethnicity, baseline health status (immunocompromised,

hereditary diseases, etc.) or any other site-specific health characteristic of the potentially exposed population that warrants special consideration.

3.6.4. Pathogen Factors

Infectivity and virulence are two pathogen factors that can also influence infection and disease (Figure 3). Infectivity is the relationship between the quantity of pathogens ingested or inhaled or in contact with skin and the probability of infection. There is probably no minimal infectious dose for enteric pathogens (Haas et al., 1999, also see Analysis Plan chapter). Virulence is a measure of the severity of the disease that the pathogen is capable of causing.

3.7. INFECTION AND DISEASE

Two primary, broad endpoints of risk assessments for pathogens in land-applied biosolids are human infection and disease (Figure 1). Infection is the process by which a microorganism multiplies or grows in or on the host. Clinical diseases are evidenced by signs or symptoms.

A variety of diseases may arise from exposure to enteric viruses (i.e., enterovirus, rotavirus, adenovirus) such as gasteroenteritis, respiratory illness, cardiovascular disease and central nervous system disorders. Likewise, the enteric bacteria associated with biosolids such as *Salmonella*, *Shigella*, C*ampylobacter*, *E. coli* and *Listeria* have been identified as causative agents of illness in exposed humans. Infections of enteric bacteria have resulted in gastrointestinal illness, dysentery, arthritis, Reiter and Guillain-Barre syndrome, and neuromuscular paralysis. The protozoans of concern *Giardia*, *Cryptosporidium* and *Entamoeba*, produce cysts and oocysts which

have been shown to be environmentally stable and somewhat resistant to disinfectants. Thus, they are recognized as significant human pathogens with the potential to cause gastrointestinal illness exhibited by diarrhea, dehydration and weight loss. Potential effects of particular pathogens in biosolids are described in the stressor characterization chapter.

Public health endpoints may include,the prevalence (total number of cases in a population) or incidence (number of new cases in a population during a specific time interval) of disease (morbidity). Mortality is an additional, potential endpoint. Severity (e.g., number of days lost to illness) may be another property of disease that is of interest to the risk assessor.

3.8. SCENARIOS

Risk assessors may describe scenarios that do not include all of the pathways in Figure 1. We consider five example exposure scenarios that represent common public concerns, and we present conceptual models for each. These do not include occupational scenarios, which are under the purview of the Occupational Safety and Health Administration. The scenarios considered here include:

1. Neighboring residences and schools adjacent to a site applied with biosolids;
2. Residents of a site where biosolids are applied (e.g., farm families);
3. Pica child playing on a site recently applied with biosolids;
4. Drinking water consumers of groundwater aquifer supplies underlying sites applied with biosolids (i.e., particularly those with highly permeable soils or shallow water tables); and

5. Drinking water consumers of surface waters downstream from sites where biosolids are applied.

3.8.1. Scenario 1. Neighboring Residences and Schools

Individuals potentially exposed to biosolids-derived pathogens may reside on lands adjacent to farms, forests, reclaimed minelands, or other lands where biosolids are applied. Similarly, schoolchildren may be exposed to eroded soils or bioaerosols from land-applied biosolids. The generic conceptual model for this scenario (Figure 4) adapts most of the pathways from the general conceptual model (Figure 1). The primary source processes that do not appear in this scenario are storage, transport and loading and unloading activities (Figure 4). For this example it is assumed that the biosolids were stored, loaded and unloaded in an enclosed facility, so exposure from these activities need not be addressed.

3.8.2. Scenario 2. Residents

Individuals potentially exposed to biosolids-derived pathogens may reside on farms where biosolids are applied. The generic conceptual model for this scenario (Figure 5) adapts all of the potential pathways from the general conceptual model (Figure 1). However, a specific model for farm families might include pathways by which biosolids-amended soil is tracked into the residence (e.g., contaminated boots, work clothes or equipment that is returned to the barn). Recreational hikers in forests where biosolids have been applied might also bring pathogens home on their clothing.

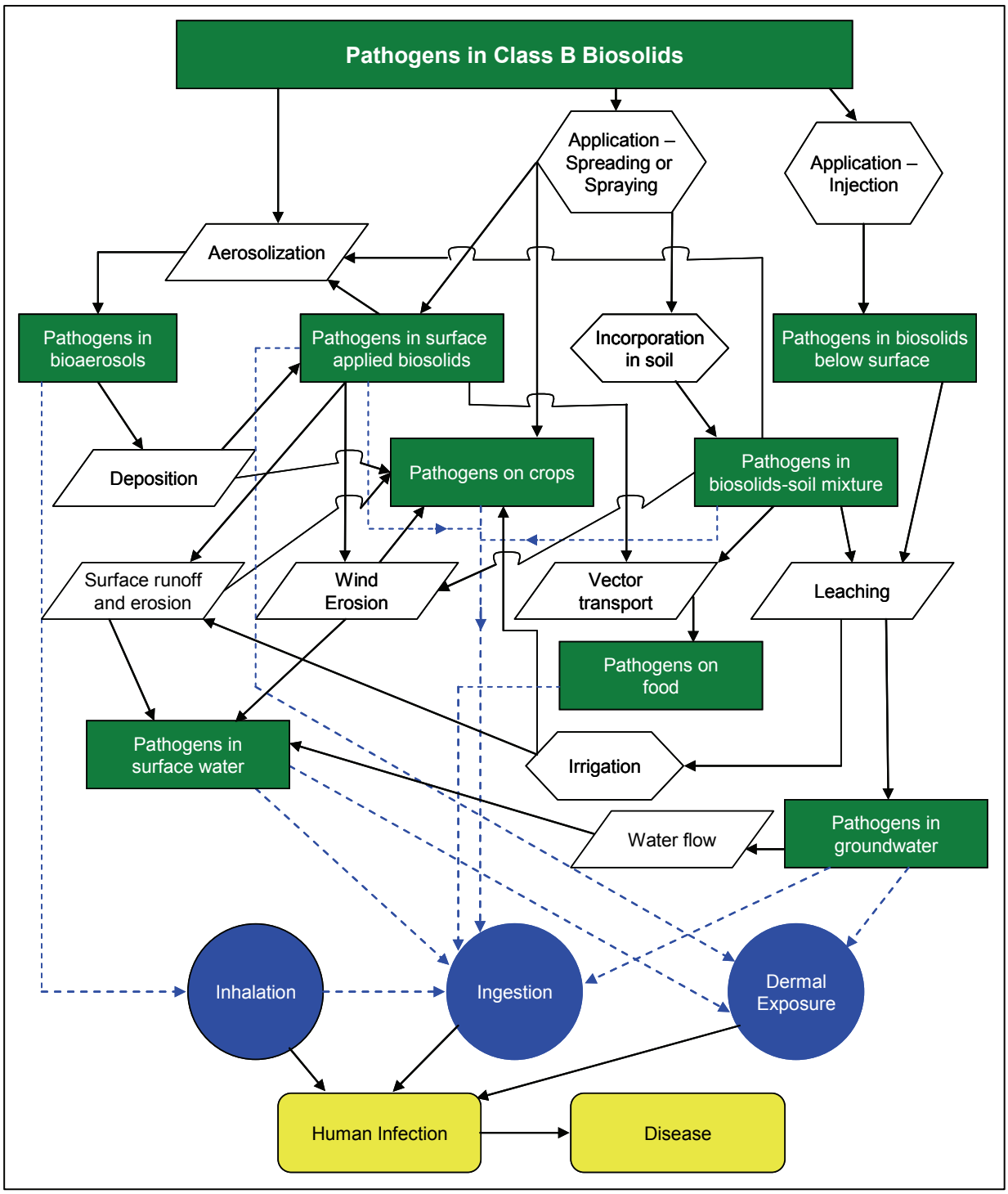

FIGURE 4

Adjacent Property Conceptual Model

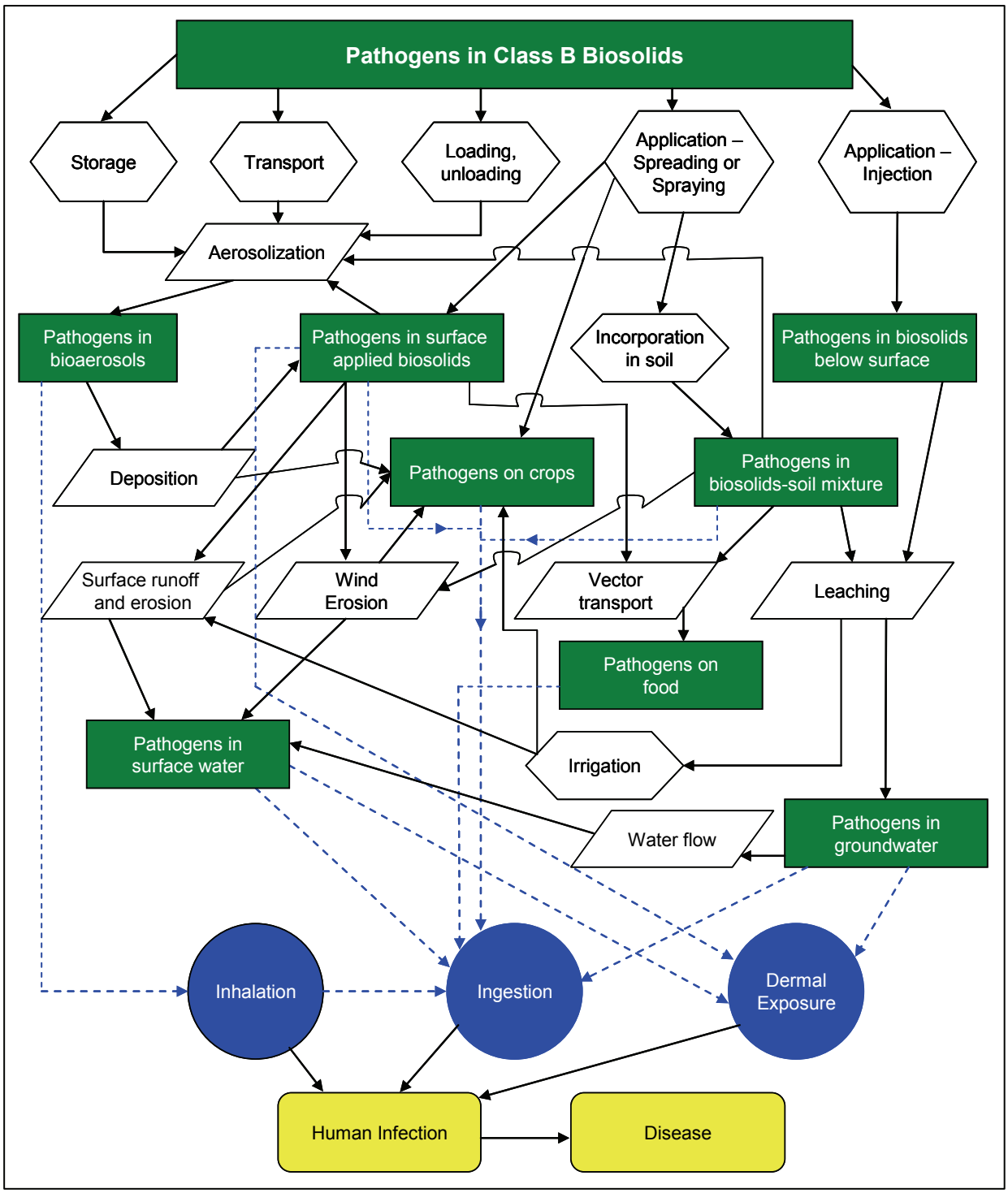

FIGURE 5

Resident Conceptual Model

3.8.3. Scenario 3. Pica Child

Soil ingestion is the consumption of soil as the result of various behaviors such as visiting treated fields and forests and consuming soil directly and indirect exposure from contacting dirty hands or contaminated crops. Moreover, soil-pica, the scenario considered here, is the recurrent ingestion of unusually high amounts of soil (i.e., on the order of 1 to 5 grams per day). Groups at risk of soil-pica behavior are generally children aged 6 years and younger. Noting that soil ingestion is a normal behavior among children, evaluation of all types of soil ingestion is included in the soil-pica scenario (Figure 6).

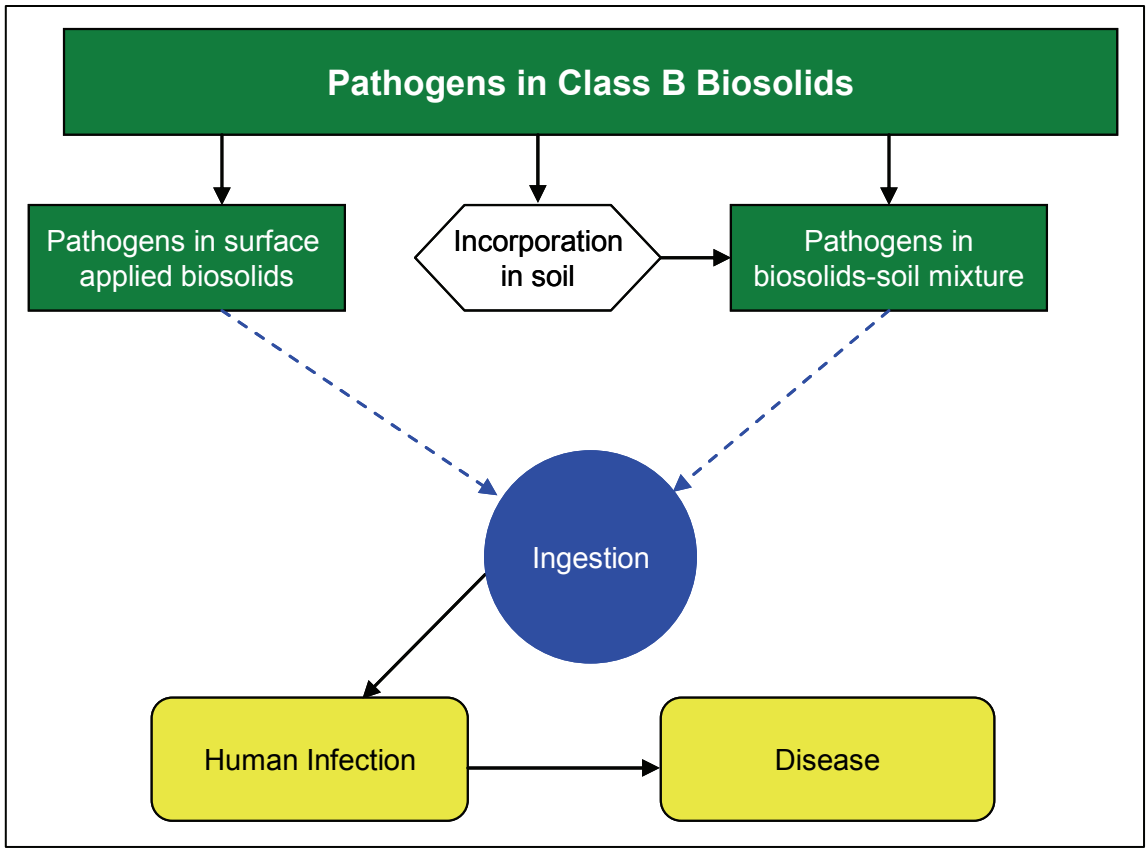

FIGURE 6

Pica Child Conceptual Model

3.8.4. Scenario 4. Drinking Water Consumers of Groundwater

Leaching to groundwater is of potential concern following injection of biosolids in the subsurface or following surface application to porous soils overlying an aquifer or well. Most drinking water aquifers contain geologic water but may be recharged following significant precipitation. Soils that are uniformly porous throughout the profile permit movement of water to aquifers or wells. Studies conducted on porous soils have demonstrated that pathogens in water can move with the liquid through soil horizons. Aquifers serve as the sole source of water in many communities and therefore may be used for both farming and domestic purposes. As such, the water may be consumed, used in food preparation (either during washing or cooking, the latter would account for significant reduction or elimination of most pathogens), bathing and other household activities. This scenario emphasizes groundwater consumption (Figure 7).

3.8.5. Scenario 5. Drinking Water Consumers of Surface Water

The use of downgradient surface waters as a source of potable water may result in exposure to biosolids-related pathogens (Figure 8). The major pathways of potential exposure to pathogens would be erosion of biosolids particles and surface runoff from treatment sites (Figure 8). Additionally, pathogens might be carried to surface water in groundwater, and small quantities of pathogens might deposit to surface waters following aerial transport. Treatment of water before consumption greatly reduces the potential for exposure.

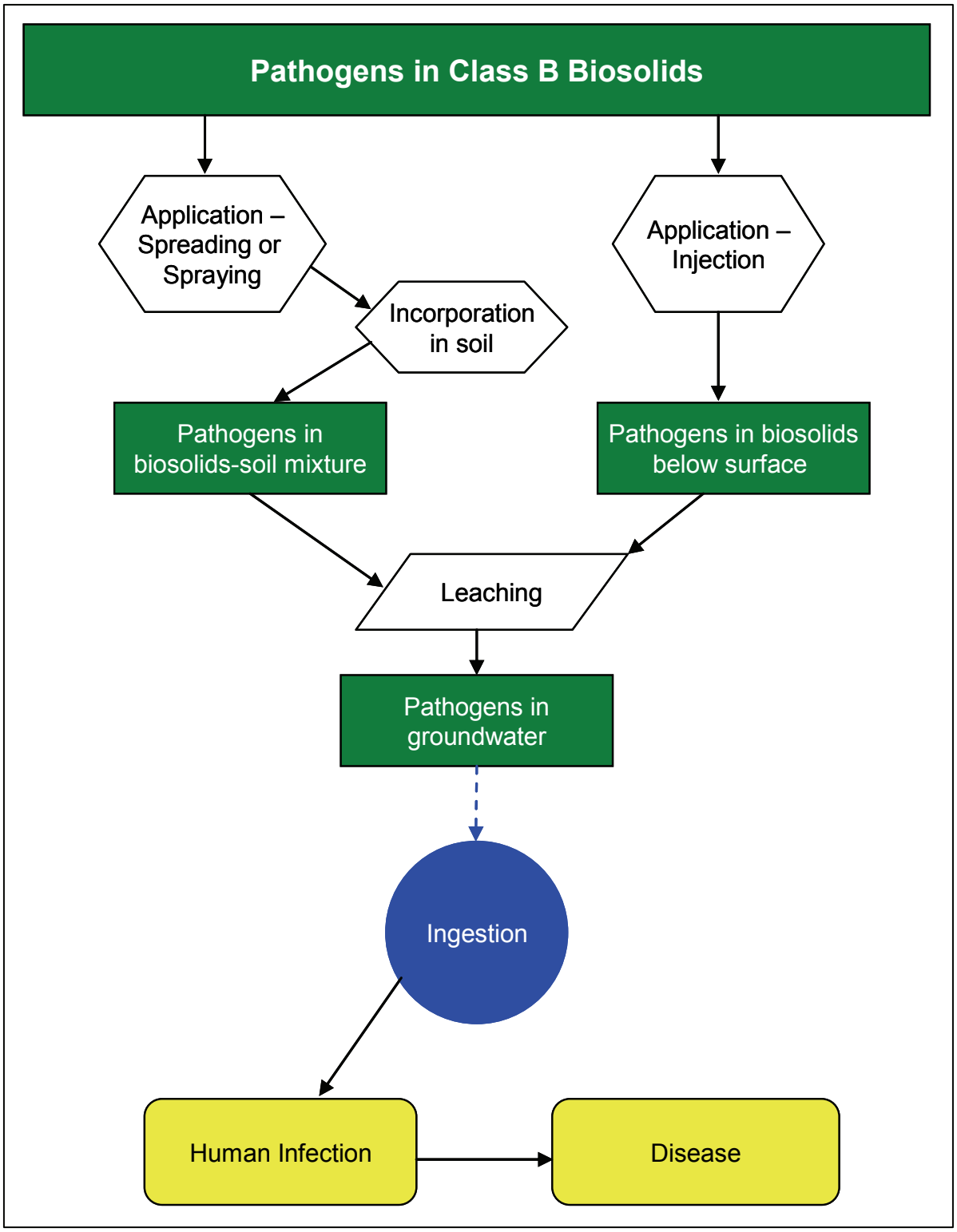

FIGURE 7

Groundwater Conceptual Model

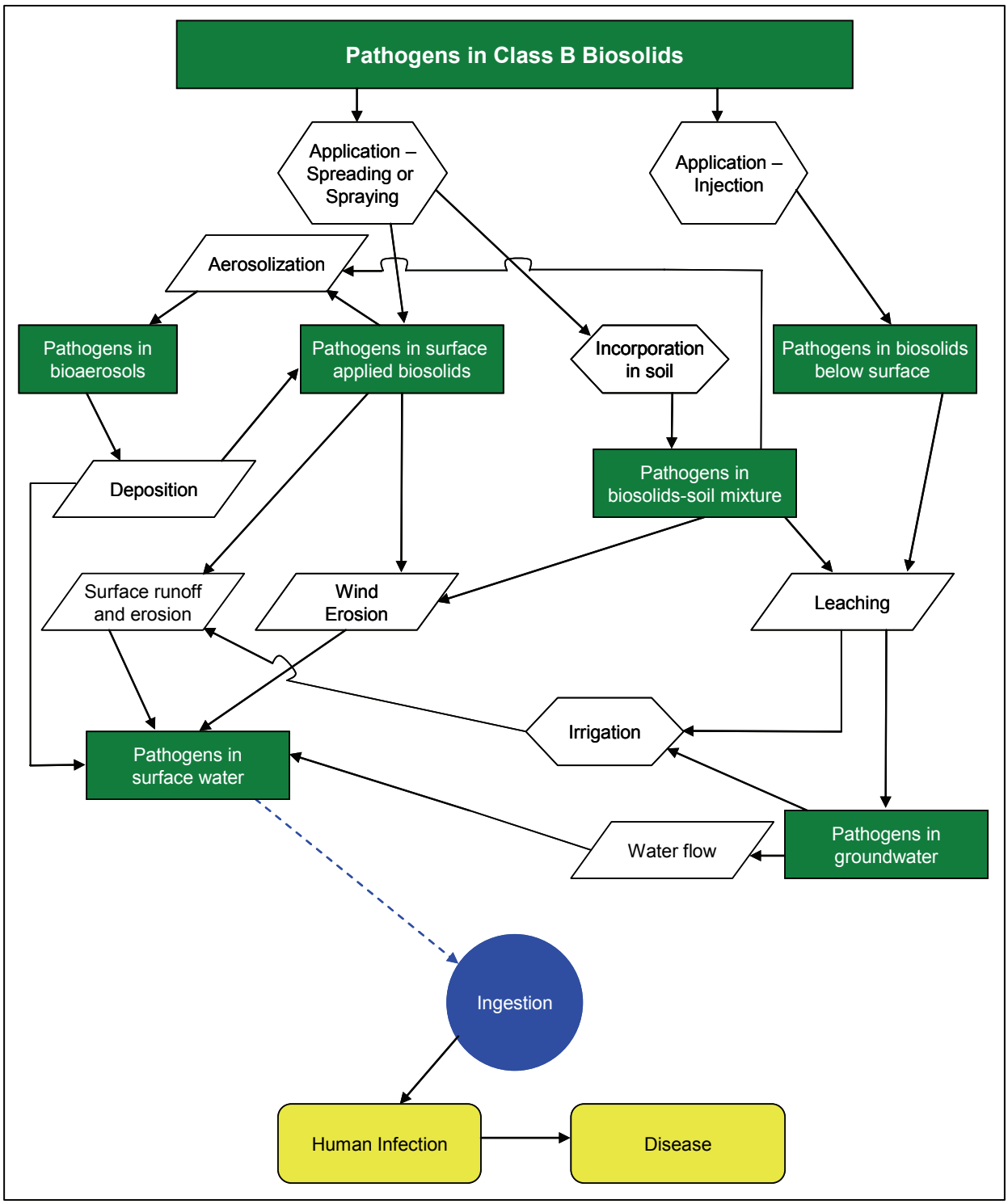

FIGURE 8

Surface Water Conceptual Model

3.8.6. Regional Aspects of Scenarios

These scenarios and others may occur in various regions. Surface water drinking scenarios would be less applicable to arid regions. Scenarios involving aerosolization of pathogens in biosolids would be more applicable to windy regions.

4. SCREENING OUT ELEMENTS OF THE CONCEPTUAL MODEL

In this chapter we examine the general conceptual model (Figure 1) to determine if sufficient information is available to screen out unlikely stressors, scenarios, routes of exposure, or endpoints from consideration in risk assessments of pathogens in biosolids. This effort should not be confused with the screening-level risk assessment process that is site-specific and part of the analysis phase rather than the problem formulation.

Very little information is available that would allow us to compare directly the relative importance of different exposure pathways. Academic studies tend to emphasize a single exposure pathway rather than a comparison of multiple pathways. However, our reading of the literature (see literature review, Appendix A) suggests that certain pathogens and exposure pathways may tend to be unimportant. However, insufficient evidence exists to support broad generalizations about negligible elements at this time.

Will this caveat in mind, risk assessors may find it easier to screen out some of the following stressors in site-specific risk assessments:

- Endotoxin. Brooks et al. (2007) found that biosolids-amended soil did not have higher levels of endotoxin than unamended soil. Levels of endotoxin in aerosolized soil were sometimes above those associated with aerosolized, biosolids-amended soil, calling into question whether biosolids were the primary source of the endotoxin (Brooks et al., 2006).

- *Staphylococcus aureus*. A broad study of 15 sites across the U.S. found that *S. aureus* was detected in raw sewage samples but not in biosolids (Rusin et al., 2003a).

- Certain protozoa. Gerba et al. (2002) determined that microsporidia and *Cyclospora* would not be likely to survive under high temperatures of anaerobic digestion or under conditions of low moisture in Class B biosolids treatment.

- Certain bacterial or viral pathogens in bioaerosols. Pathogens and indicator bacteria were only rarely found in aerosolized samples in a study of land application of biosolids in Tucson, AZ. These included coliforms and coliphages, which were present at high densities in biosolids. The authors suggested that only microorganisms in the aqueous phase of biosolids were able to aerosolize; others remained sorbed to the solid phase (Brooks et al., 2004). Furthermore, Tanner et al. (2005) determined bioaerosol emission rates and plume characteristics during spray application of liquid Class B biosolids. They did not detect coliphages or coliform bacteria just downwind of the biosolids application, though pathogens sprayed in inoculated groundwater were detected. The researchers concluded that the presence of biosolids reduces aerosolization of microorganisms relative to application of inoculated groundwater. The duration of exposure to any pathogens (below detection limits) downwind of biosolids application is brief (Tanner et al., 2005).

Brooks et al. (2005b) undertook a study to estimate risks of microbial infection of residents near biosolids application sites. At 10 sites (five in Arizona, five elsewhere in the U.S.) amended with either liquid or solid Class B biosolids, they measured heterotrophic plate counts (HPC) bacteria, total coliform bacteria, *E. coli*, *Clostridium perfringens*, coliphage, enteroviruses, hepatitis A virus and norovirus in aerosol samples downwind from application sites. The study distinguished between loading, unloading, land application and background operations. In general, risks of infection were determined to be low, with the greatest risks, that of infection by coxsackievirus A21 from loading operations having a 4×10^{-4} chance of infection. Based on this work, Pepper et al. (2006) concluded that the overall community risk of infection from bioaerosols during land application was relatively negligible.

Some evidence (below) might support a decision to screen out certain exposure pathways (Figure 1) from general or regional consideration in the future. However, more evidence is needed to support such a judgment.

- Groundwater pathway. Because of the large size of bacteria, soil (especially fine-textured soil) can act as a filter to limit bacterial transport (NRC, 2002). Soil would also be expected to limit the transport of larger protozoa and helminths (NRC, 2002). A review of the literature has concluded that few pathogens (even viruses) from biosolids leach to groundwater (Pepper et al., 2006). Although Gerba (2005) acknowledges that of the pathogens in biosolids, viruses have the greatest potential for contamination of groundwater, Pepper et al. (2006) concluded that "groundwater contamination from land-applied biosolids does not appear to be likely." Sandy soils with low cation exchange capacity deserve more study.

- Root crop ingestion pathway. A United Kingdom study of infection from consumption of root crops grown on biosolids-amended soils found that risks to humans was low. Seven pathogens were included in the study: salmonellas, *Listeria monocytogenes*, campylobacters, *Escherichi coli* O157, *Cryptosporidium parvum*, *Giardia* and enteroviruses (Gale, 2005b). United Kingdom biosolids may not be comparable to Class B biosolids in the U.S.

Regulations might also allow a risk assessor to screen out potential pathways of exposure in the general case. For example, if biosolids must be stored in enclosed facilities, the generation of bioaerosols from that source (and exposure to neighboring residents) would not be likely.

5. ANALYSIS PLAN

5.1. INTRODUCTION

The analysis plan is the final stage of problem formulation. It summarizes the measures, methods and data needs for conducting the analysis phase of the risk assessment, i.e., the characterization of exposure and the characterization of effects. Methods are described to characterize the source, pathways, environmental media and human endpoints. The emphasis is on variables to which the risk assessment is sensitive, if known. A rigorous analysis plan is especially necessary if there is no established protocol for conducting a particular type of risk assessment (U.S. EPA, 1998), as with human health risk assessment of biosolids-derived pathogens.

The analysis plan evaluates risk hypotheses to determine how they will be assessed (U.S. EPA, 1998, 2003a). The rationale for selecting or eliminating risk hypotheses is set forth (U.S. EPA, 1998). An analysis plan for a risk assessment of pathogens in biosolids must be designed to eliminate negligible pathways in the conceptual model. Available data are described, as well as new data that should be collected to conduct the risk assessment and the feasibility of their collection. The analysis plan describes both measurements and models. The plan also describes where parameters of interest may be extrapolated from existing data. Extrapolation allows the use of data collected from other locations or for other microbial pathogens where similar problems exist.

This chapter is structured as an analysis plan might be structured for a risk assessment on land-applied biosolids. Following the introduction, we discuss management needs, including parameters requiring estimation and data quality

objectives. Then we discuss the plan for the characterization of exposure, including the selection of measures of exposure, the detection of microbes, the issue of background levels of pathogens and the estimation of fate, transport, uptake and dosage. The plan for the characterization of effects follows, including the selection of measures of effect, establishing cause and effect and dose-response models for infection. Methods for predicting disease, including the existence of thresholds and the role of immunity and epidemiological methods are also discussed. Finally, the plan for risk characterization is set forth, including the issue of standards, the possibility of tiered analysis, the weight-of-evidence approach, probabilistic assessment and uncertainty analysis.

The emphasis in this chapter is on aspects of analysis plans that are unique to risk assessments for biosolids-derived pathogens rather than risk assessments for pathogens in general. Therefore, some of the dose-response and epidemiological information is deemphasized. Furthermore, because of the numerous research gaps, we identify research, observational studies and methods development that should be performed to complete a defensible risk assessment to support regulatory actions. Finally, because this is a generic framework for an analysis plan, it does not contain the level of detail that would be expected in an analysis plan for a specific site or a particular regulatory action. This report does not provide site-specific advice on how to prioritize data needs, models or assessment endpoints.

5.2. MANAGEMENT NEEDS

Risk mangers have two fundamental requirements of risk assessors. The assessment process must estimate risks to endpoints that are important to the decision, and the results must have sufficient quality to be reliable.

5.2.1. Assessment Endpoints

In any risk assessment, the assessment endpoint is an explicit expression of the value that should be protected. In health assessments, the endpoint is a property of human health. Many risk assessments for pathogens in biosolids will be conducted by U.S. EPA's Office of Water, and therefore, risk managers from this office will determine the appropriate assessment endpoints. These may include population-level endpoints or individual-level endpoints. It may be desirable to estimate the probability of infection (individual endpoint), number of infections during a period of time (population endpoint), number of infections during an outbreak (population endpoint), disease incidence (population endpoint), or related endpoints. The endpoint may be cumulative (estimating risk from pathogens of all sources) or may focus on only those infections or illnesses that are estimated to result from pathogens in biosolids. The risk manager may also specify levels of infection or disease that are acceptable or that require regulatory action. If applicable, these levels, as well as other properties of the assessment endpoint, should be described in the analysis plan. A purpose of the analysis plan is to set forth methods for estimating the assessment endpoint. The assessment endpoints will allow U.S. EPA to determine the level of public health and environmental protection from pathogens in biosolids afforded by 40 CFR 503, determine protective buffer distances, or validate the current operational standards and management practices.

5.2.2. Data and Data Quality

U.S. EPA (1998) recommends that risk assessors ask several general questions related to the selection of data for the assessment:

- How relevant will the results be to the assessment endpoint(s) and conceptual model(s)?
- Are there sufficient data of high quality to conduct the analyses with confidence?
- How will the analyses help establish cause-and-effect relationships?
- How will results be presented to address managers' questions?
- Where uncertainties are likely to become a problem?

The analysis plan also specifies data quality objectives for the risk assessment. The Superfund program provides a good model for specifying the type of information that is needed to ensure data quality, specifying necessary and optimal levels of data quality, and identifying the means of obtaining this information from risk managers (U.S. EPA, 1994). These steps are described in Text Box 1.

5.3. PLAN FOR CHARACTERIZATION OF EXPOSURE

5.3.1. Measures of Exposure

The first step to planning the characterization of exposure is selecting the measures of exposure. Measures of exposure are measures of stressor existence and movement in the environment and their contact or co-occurrence with the assessment endpoint entity. More specifically, in a human health risk assessment these are measurable characteristics of pathogens that are used to quantify exposure of humans

or contact with particular organ systems. Measures of exposure include concentrations of particular pathogens in environmental media or components of these media (biosolids, biosolids-amended soil, air, water, clay, aerosols). Measures of exposure to microbial pathogens may also include inputs to models of fate, transport, or exposure (e.g., doses to humans), as described below.

Text Box 1.
Recommended Steps for Specifying Data Quality Objectives (modified from U.S. EPA, 1994).

1. State the Problem. Clearly specify the question that relates to pathogens in biosolids. Is the concern a generic national problem? Or is it a site-specific one? Has an infection or disease been observed where the cause is unknown? Or is the risk manager concerned with future prediction?
2. Identify the Decision. Identify the decision that must be made to solve the problem. For example, are new regulations required to prevent unacceptable risk to human health?
3. Identify Inputs to the Decision. Identify the information needed to make the decision and measurements, simulations, and other analyses that must be undertaken to provide that information. These are the major components of the analysis plan.
4. Define the Assessment Boundaries. Specify the conditions to be assessed, including the spatial area, the time period and the exposure scenarios to which the decision will apply and for which inputs must be generated.
5. Develop Decision Rules. Define conditions under which an action, such as the promulgation of new regulations, will be taken.
6. Specify Acceptable Limits of Decision Error. Define error rates that are acceptable to the risk manager.
7. Optimize the Design. Design a study in which new data are collected and design the use of existing data in exposure or effects models, such that the expected variance in parameters results in an acceptable limit in decision error.

5.3.2. Detection of Pathogens

Following the selection of measures of exposure, the detection of pathogens is the first type of analysis required in the analysis plan. As stated in the literature review (Appendix A), one of the major data gaps related to pathogens in biosolids is a recent national survey regarding levels of particular pathogens in sewage sludge and biosolids. Appropriate analytical methods are also needed for detecting and quantifying particular pathogens in sewage sludge and biosolids. This information is needed to support

national-scale human health risk assessments of biosolids. In site-specific risk assessments, it is possible to analyze the biosolids, amended soil, water, air or bioaerosol of concern to estimate pathogen levels, though these methods have high levels of uncertainty. The only current option for national scale risk assessments is to conduct analysis of pathogens in biosolids at several application sites that are thought to be representative of such sites across the country.

5.3.2.1. *Bacteria*

Smith et al. (2005b, Chapter 4) describe detection and enumeration capabilities for bacterial pathogens that involve general or selective enrichment combined with selective culturing or polymerase chain reaction (PCR) and molecular identification techniques. However, these experts acknowledge that the use of these methods to detect all potential pathogens in a sample might be too costly or require too much effort to be practical. Thus, the use of indicator organisms is recommended if adequate indicators and appropriate analytical methodology are available (Smith et al., 2005b, Chapter 4) (see section on *Use of Indicator Species* below). Recent research on species-specific biosensors may also produce useful products for detecting pathogens in biosolids (e.g., Guntupalli et al., 2007).

Organic matter and high bacterial counts reduce recovery fraction for pathogens in biosolids or amended soils (Rusin et al., 2003b). The analysis plan should indicate the recovery rates for the detection technologies that will be used. For example, recovery percentages of bacterial pathogens in aerosols that are reported in the literature are currently about 10% (Lubick, 2007). Rusin et al. (2003a) had a recovery

efficiency of 8.7% for *Staphylococcus aureus* in Class B biosolids. U.S. EPA has new standardized analytical methods for fecal coliforms and *Salmonella* (FR 57 14219).

5.3.2.2. *Viruses*

Sampling and detection of viruses that are present at high levels in biosolids is much easier than demonstrating conclusively that viral agents are not present (NRC, 2002). The primary determinant of the ease of detection of viruses is whether they can be cell-cultured. Of the viral pathogens listed in the stressor characterization chapter, astroviruses, rotaviruses, hepatitis A and E and adenoviruses can be cell-cultured, whereas human caliciviruses cannot (NRC, 2002). Methods used to recover viruses from sewage sludge have been optimized for the enteroviruses rather than for other enteric viruses (Goyal et al., 1984; Gerba and Smith, 2005). Therefore, risk assessors need to be aware that there is high uncertainty regarding concentrations of non-enteroviruses in raw sewage sludge and treated biosolids (Smith et al., 2005b, Chapter 8). And risk assessors should indicate in the analysis plan that risks from caliciviruses cannot be determined at this time. Disadvantages of cell culture methods include the high cost, long time required for positive results (up to one month) and the presence of potentially toxic organic compounds and inorganic elements in sewage sludge.

PCR is an alternative family of methods for identifying viruses. These analyses are quick, relatively inexpensive and sensitive. Direct reverse transcriptase PCR (RT-PCR) detects nucleic acid sequences from active and inactive viral particles, and thus may overestimate exposure. Integrated cell-culture PCR (ICC-PCR) amplifies viruses in cell culture and amplifies viral RNA through enzymatic PCR. ICC-PCR is the recommended method for viral risk assessment because of the potential for cell culture

alone to underestimate human exposure and for RT-PCR to overestimate exposure (NRC, 2002).

5.3.2.3. *Helminths*

Various assays for helminth eggs in biosolids are available, but no standard assay exists, mainly because quality-assurance and quality-control studies have not been published for many study protocols (NRC, 2002). Candidate methods are referenced in NRC (2002), each with different recovery percentages for *Ascaris* eggs. Many do not adequately consider sample preservation and pretreatment. Some of these are not very accurate. The Tulane assay is discussed with recovery percentages, but this assay may not be valid for detecting helminths such as *Trichuris trichiura* that have eggs of different densities from *Ascaris* (NRC, 2002).

5.3.2.4. *Protozoa*

Methods for detecting helminths may be applicable to protozoa if final sieve size is adjusted to the smaller size of *Giardia* and *Cryptosporidium*. Viability and infectivity assays for protozoa that are available for the analysis plan include vital dye staining, animal infectivity, cell culture or PCR. Recoveries from biosolids are low, e.g., 10% for the sedimentation technique, less than 3% for the flotation technique, 3.2-16.3% for *Cryptosporidium* oocysts and 2.4-41.7% for *Giardia* cysts (NRC, 2002).

5.3.3. Use of Indicator Species

Because of the wide range of pathogens found in human feces, domestic wastewater and biosolids, direct monitoring and quantification of all of the pathogens in

biosolids may not be practical for a site-specific risk assessment (Nappier et al., 2006). Indicator species are abundant and typically non-pathogenic microorganisms that may be used to indicate the presence of a suite of pathogens. For example, fecal coliform density and *Salmonella* are used as indicators of wastewater treatment efficiency (40 CFR 136). Tests for indicator microorganisms should be relatively simple and routine (NRC, 2002). However, most indicators have been chosen to indicate treatment effectiveness rather than measures of pathogens that are quantitative and are more closely related to public health (Smith et al., 2005b, Chapter 4). Tanner et al. (2005) cite research in their laboratory and other literature to show that (a) there is approximately one human pathogenic bacterium per 1000 coliform bacteria in biosolids and (b) one human enteric virus in Class B biosolids per 1000 coliphage. However, this estimate is not helpful for pathogen-specific risk assessments, because the identity of the pathogen is an important determinant of risk.

Bacteria and helminths. Indicators of a range of pathogens in biosolids are needed. It may not be feasible for individual risk assessors to develop these indicators in the analysis plans for individual risk assessments. Given the resistance of spore-forming bacteria to desiccation, indicators of these bacterial pathogens would need to behave similarly. The NRC (2002) discusses *Clostridium perfringens* as a potential indicator of the efficiency of disinfection. In particular, they provide references suggesting that its spores might be a surrogate for eggs of *Ascaris suum* because of its resistance to similar chemical and physical disinfection agents. Furthermore, Dowd et al. (1997) recommend thermotolerant clostridia as indicators of fecal contamination in bioaerosols. Pillai et al. (1996) found that clostridia and H_2S producers were detected

on glass impingers at locations near biosolids-amended sites where traditional bacterial indicators (fecal coliforms and fecal streptococci) were not. Thus *Clostridium perfringens* may be a useful surrogate for a range of pathogens in the analysis plan. Risk assessors may consider indicators of anaerobic pathogens, but genera such as *Bifidobacterium* and *Bacterioides* cannot be reliably detected and therefore cannot be routinely monitored (NRC, 2002).

Viruses. Smith et al. (2005b, Chapter 5) summarize the suitability of selected agents as indicators of treatment performance and post-treatment risk for viruses. Only the latter is relevant here and is presented in Table 7. Bacteriophages are the only potential indicator viruses mentioned in NRC (2002) because of their presence in sewage. Because somatic coliphage infects strains of *E. coli*, it can be detected using simple, inexpensive methods (NRC, 2002). Lime is also included as a potential indicator of post-treatment risk for viruses in Smith et al. (2005b), presumably because enteric viruses should be eliminated with extended alkaline treatment. At this time, these indicators are qualitative. Risk assessors would need to do substantial testing to quantify relationships between these indicators and pathogens of potential concern.

5.3.4. Background Levels of Pathogens

The analysis plan should assess background levels of pathogens through measurement or extrapolation from regional values if available. Background levels of pathogens are levels in environmental media (soil, water or air) not amended with or contaminated by biosolids. Background levels are due to colonization of media at the regional scale. For example, endospore-forming bacteria such as *Clostridium*

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TABLE 7

Suitability of Select Agents as Indicators of Post-Treatment Risk for Viruses in Biosolids, Modified from Smith et al. (2005b)

Agent	Suitability
Adenoviruses	?
Ascaris	yes
Coliphages	yes
Clostridium perfringens spores	yes
Enterococci	no
Enteroviruses	yes
E. coli	no
Fecal coliforms	no

perfringens are very common in soil. The risk assessment is only concerned with the incremental risk from pathogens in biosolids or the cumulative risk from pathogens in biosolids-amended soil, rather than the risk from pathogens in soil alone.

Background levels of pathogens may be significant contributors to risk. For example, in a study of aerosolized endotoxin concentrations downwind from a biosolids-amended site, Brooks et al. (2006) found that levels of endotoxin in aerosolized soil were sometimes above those associated with biosolids amended-soil, calling into question whether biosolids were the primary source of the endotoxin.

5.3.5. Environmental Fate of Pathogens

The survival or regrowth of pathogens should be estimated if the risk assessment is prospective (i.e., concerned with forecasting), and environmental media cannot be sampled at the time of interest. Regulations that limit contact with biosolids do not prevent environmental processes in the conceptual model such as aerosolization or erosion (Figure 1) and the death or multiplication of pathogens (Figure 2). Therefore, the analysis plan may include a plan for estimating pathogen fate. Most models of the fate of pathogens in sewage sludge are concerned with predicting the reduction or inactivation of pathogens by treatment processes (e.g., Epstein, 2006). Straub et al. (1993) reviewed available studies of survival of pathogens in soil and sewage sludge that are pertinent to this analysis plan discussion. Gerba and Smith (2005) provide survival times of pathogens on soil and plants (Table 8).

Risk assessors should not use survivorship data from enteric organisms such as *E. coli* and *Salmonella* to estimate the much longer survival rates of bacterial pathogens that form spores or are encapsulated (such as *Mycobacterium* spp.).

TABLE 8

Survival Times of Pathogens in Soil and on Plants
Modified from Gerba and Smith (2005)

Pathogen	Soil		Plants	
	Absolute Maximum	Typical Maximum	Absolute Maximum	Typical Maximum
Bacteria	1 year	2 months	6 months	1 month
Viruses	6 month	3 months	2 months	1 month
Protozoa	10 days	2 days	5 days	2 days
Helminths	7 years	2 years	5 months	1 month

5.3.6. Transport of Pathogens

The conceptual model in Figure 1 describes several transport processes, including wind erosion, surface runoff and water erosion, aerial dispersal of bioaerosols, deposition on crops, leaching to groundwater and vector transport. The analysis plan needs to provide a plan for answering the questions of how far and in what concentrations pathogens will travel. Models are available for most transport processes, though they have some limitations.

5.3.6.1. *Water Erosion*

Water erosion is typically modeled using the universal soil loss equation or its modifications. Average annual soil erosion is the product of a rainfall erosivity index, soil erodibility factor, topographic factor, cropping factor and conservation practice factor

(Wischmeier and Smith, 1978). The soil erodibility factor estimates the cohesive nature of a soil type and resistance to transport from raindrop impact and surface flow. While this factor is available for various soil types, to our knowledge it has not been measured for biosolids or biosolids-amended soils. The crop management factor is specific to agricultural systems and can include tillage but could be adapted to forest, greenway, mineland, or other biosolids application sites. Significant soil disturbance resulting from tracked vehicles could be incorporated in the soil erodibility or crop management factors. A limitation is that this equation is not applicable to a specific storm or year. If erosion is expected to be a significant transport process, these analyses would need to be part of the analysis plan.

5.3.6.2. *Surface Runoff and Aqueous Transport*

Methods for estimating surface runoff should be described separately from erosion models in the analysis plan. For example, Montemagno et al. (2004) describe a modeling strategy for estimating surface water contamination by pathogens from agricultural sources, using the specific example of oocysts of *Cryptosporidium*. Both surface runoff and water erosion are simulated.

For site-specific assessments, it may be desirable to use a spatially explicit model to simulate transport from land to streams and through a watershed to recreational areas or water intakes. BASINS (http://www.epa.gov/waterscience/basins/) provides an integrated system for such assessments. Alternatively, simple models of dilution and transport in a generic stream can be used.

5.3.6.3. *Wind Erosion*

Wind erosion should be considered in areas where wind speeds are often above the 19.3 km/h required to initiate soil movement (Brady, 1974). Wind erosion is controlled by 11 primary variables: soil erodibility, knoll erodibility, surface crust stability, soil ridge roughness, wind velocity, surface soil moisture, distance across field, sheltered distance, quantity of vegetative cover, kind of vegetative cover and orientation of vegetative cover (Woodruff and Siddoway, 1965). The Wind Erosion Equation, developed by Woodruff and Siddoway (1965) groups many of these variables and is a function of the erodibility factor (which increases with percentage of soil particles greater than 0.84 mm diameter), a ridge roughness factor, a climatic factor, a field length factor and a vegetative cover factor. Clearly, the erodibility factor would be specific to biosolids, but the climatic factor, which incorporates soil moisture, would also be affected by biosolids added to the surface of soil or incorporated in soil. Again, this equation is not applicable to a specific year or wind event. Also, the Wind Erosion Equation provides a measure of dislodged soil; the equation provides no estimates of the travel distance of the soil (Batie, 1983).

5.3.6.4. *Aerial Transport of Bioaerosols*

To estimate bioaerosol transport, a risk assessor must understand the release rates of the different microbes, the dispersion of the bioaerosols and the deposition of the microorganisms (Pillai, 2007). These quantities depend on whether pathogens are aerosolized during particular types of biosolids application or following application. Pathogens in bioaerosols and their transport may be measured or modeled. The

analysis plan may include measurement of pathogens in air as a source term for a dispersion model or near the human receptors of interest.

The sampling of bioaerosols involves the removal and concentration of biological particles from the air (Pillai and Ricke, 2002). Sampling bioaerosols poses a particular challenge, compared to sampling of biosolids. Impaction, impingement, gravity settling, filtration and electrostatic precipitation are options for concentrating microorganisms from bioaerosols, but efficiencies of collection can be low or uncertain (NRC, 2002; Pillai and Ricke, 2002). Where molecular assays are feasible, collection methods do not have to preserve the viability of microbes, as they did when culture methods were required for identification (Pillai and Ricke, 2002). Although there is a standard method for assessing occupational exposures to bioaerosols in indoor environments, no comparable standard exists for outdoor environments, and some of the indoor samplers that rely on external vacuum and power sources cannot be carried to remote sites (NRC, 2002). Insufficient testing of available methods has occurred to recommend a particular sampling method for bacteria in bioaerosols, but we recommend that assessors describe methods for testing sampling efficiencies of their equipment in the analysis plan. Risk assessors should also be aware that during transport, deposition and sampling, bacteria can be desiccated or inactivated, resulting in failure to culture and an underestimation of the number of viable cells. The analysis plan should specify how sampled pathogens will be handled.

Furthermore, determining an appropriate spatial distribution of samples is a challenge for sampling bioaerosols. If tens of acres are amended with biosolids, substantial micrometeorological differences may result from differing topography,

vegetation and mechanical agitation (NRC, 2002). Wind direction and speed may vary during the sampling time. The orifices of bioaerosol samplers downwind may be too small to obtain detectable levels of bacteria, even if they are present in bioaerosols. Thus, appropriate statistical analysis (Spicer and Gangloff, 2000) and appropriate numbers of replicates are uncertain. These issues should be addressed in the analysis plan.

Models are available to estimate transport of pathogens in bioaerosols (Dowd et al., 2000; Brooks et al., 2005a). "Point-source" transport models are appropriate for localized sources of biosolids, such as a storage pile, and "area-source" models are more appropriate for predicting concentrations of pathogens downwind from a large biosolids-amended field in which including the length and width of the field more accurately estimates aerosol loading rates (Dowd et al., 2000). Dowd et al. (2000) modified a standard point-source transport model to incorporate the expected reduction in microbial concentration with increased distance from the source. Variables included the inactivation rate of the microorganism, mean wind speed, diffusion constants, downwind distance from source and height of sample. Typically, the risk assessor needs to back-calculate the rates of release of microorganisms from the source using sampling data, because measurement is extremely difficult (Dowd et al., 2000).

An empirical model is another option for estimating aerosolized pathogen concentrations with distance from the source. Brooks et al. (2005a) derived a linear regression model that estimated coliphage concentrations at various distances from the spray application location, normalized for initial microbial concentration and wind speed. The researchers conducted field tests with coliphage MS-2 added to water and sprayed

with a biosolids spray application truck. Temperature was also observed to influence aerosol concentration (Brooks et al., 2005a). The relationship these researchers derived may not be applicable to other biosolids, application methods or regions, but the development of similar empirical models may be an objective of the analysis plan.

Correlations have been developed between microbial levels in biosolids and their concentrations emitted during disking (Paez-Rubio et al., 2006) and spreading with a slinger side-spreader (Paez-Rubio et al., 2007). These types of reconstructions permit risk assessors to avoid difficulties of detecting pathogens in aerosols.

Indicator species may be used to estimate transport of related pathogens. For example, the ratio between the concentration of indicator virus in aerosols and the concentration in biosolids was used to estimate a value for airborne enteric virus (Coxsackievirus) in Dowd et al. (2000).

Even allowing for sampling limitations and recovery efficiency issues, measurement is probably superior to models (which are validated using measurements in any case). Many of the physicochemical interactions between pathogens and biosolids and between pathogens and other components of bioaerosols are difficult to model. For example, viruses have been observed to sorb strongly to biosolids particles but to aerosolize more easily if present in the liquid fraction of biosolids (Brooks et al., 2004). The transport of large dust particles is not usually modeled. Moreover, during application, the aerosol plume at each location is detectable for only a short period of time (e.g., less than one minute per pass of a spray applicator in Tanner et al. [2005]). Complicating factors include variation in terrain, topography, vegetation, micrometeorological conditions, biosolid composition and biosolids land application

processes (Pillai, 2007). Also, the bioaerosol transport reconstruction in Paez-Rubio et al. (2006) tended to result in a lower concentration than what was measured. Thus, risk assessors should justify the use of particular models in the analysis plan.

5.3.7. Contact with Crops

Pathogen residues on root and leaf crops can be measured. Biosolids and associated pathogens can deposit to crop leaves following erosion, aerial transport or rainsplash, and these processes can be modeled. Because of the ease of measurement and uncertainty of modeling, we recommend that pathogens on select crops be measured. If measurement is not possible, risk assessors can estimate the biosolids residues on root and leaf crops based on standard crop exposure assumptions (U.S. EPA, 1997), though these assumptions do not account for aerosolized pathogens depositing directly on leaves. Gale (2005b) offers assumptions that 10% of root crops were consumed unwashed or that 90% of soil was removed by washing prior to consumption.

Gale (2005a,b) describes ramifications of using the arithmetic mean root crop concentration as an input to dose-response models. This statistic often overestimates the number of people who are exposed to pathogens, because where pathogens are spatially clustered, many individuals are not exposed. Thus, the analysis plan should indicate that the arithmetic mean exposure concentration (if used) may give a conservative estimate of the number of people exposed.

5.3.8. Uptake and Dosage

The analysis plan should include methods for estimating inhalation, ingestion and dermal exposure when consideration of those routes of exposure is appropriate (see conceptual model discussion). For example, the dose of aerosolized pathogens to a person during a period of time may be estimated by measuring or modeling concentrations of microbes at a specific distance from the source and the inhalation rate over a period of time.

5.3.9. Exposure Factors

U.S. EPA does not have standard exposure factors for use in risk assessments of pathogens in biosolids. However, many of the exposure factors and assumptions described in the *Exposure Factors Handbook* (U.S. EPA, 1997), which was designed for use in human exposure assessments for chemical contaminants, are pertinent. These include general exposure factors (e.g., drinking water intake rates, soil ingestion rates including for the pica child scenario, inhalation rates, body weight, body surface area), food ingestion factors (e.g., fruit and vegetable intake rates and water contents) and activity factors (e.g., time spent outdoors). This and other risk assessment guidance is available from the Risk Assessment Information System (U.S. DOE, 2006).

Some of the exposure factors in U.S. EPA (1997) may not be pertinent to risk assessments for pathogens in biosolids. For example, activity factors that estimate time spent outdoors may not be as relevant for a risk assessment of bioaerosols generated during biosolids application as the duration of the application process. The percentage of inhaled particles that would be ingested should be specific to biosolids-generated

aerosols. Pepper et al. (2006) describe studies that use a factor of 10%, and Brooks et al. (2005b) uses 50%. Haas et al. (1999) recommend exposure factors that are relevant to risk assessments for pathogens. While many of these factors are analogous to those in U.S. EPA (1997), others are more pertinent to risk assessments for pathogens (e.g., proportion of pathogens that are transferred to and from hands).

5.4. PLAN FOR CHARACTERIZATION OF EFFECTS

5.4.1. Measures of Effect

A measure of effect is a measurable quantity that is used to estimate the effects of exposure (to biosolids-derived pathogens) on the assessment endpoint. In this problem formulation, assessment endpoints include aspects of human health estimated at the individual level or population level. The analysis plan describes the measures of effect for the risk assessment. Suter et al. (2000) summarized considerations in selecting measures of effect for ecological risk assessments of chemical contaminants. These considerations are adapted here for pathogens in biosolids.

- Corresponds to an assessment endpoint
- Relates to the human health endpoint in a quantifiable manner
- Makes use of existing data
- Is readily measured
- Is of appropriate temporal and spatial scale
- Is appropriate to the exposure pathway
- Is appropriate to the mode of action
- Is diagnostic of particular pathogens
- Shows low variability, increasing the likelihood of detecting an effect
- Is broadly applicable to different locations
- Is a standard test or measurement method

The first two considerations are necessary to meet the definition of a measure of effect.

Measures of effect are derived from laboratory studies (e.g., rat or mouse ingestion or bioaerosol inhalation studies) or epidemiological studies designed around biosolids application or disease outbreaks (controlled human clinical studies involving ingestion or inhalation are likely rare or nonexistent). Studies of disease outbreaks are often used to validate measures derived from animal models. The most applicable data would come from studies with biosolids, but other studies of pathogens can provide relevant data, especially in the absence of studies of biosolids.

Measures of effect in this problem formulation for biosolids-derived pathogens may include probability of infection (individual measure), number of infections during a period of time (population measure), number of infections during an outbreak (population measure), disease incidence (population measure) or related measures.

5.4.2. Establishing Cause and Effect

As noted in the literature review (Appendix A), a causal association between exposures to pathogens in biosolids and adverse effects on human health has not been documented. Risk assessors should examine relevant data (and perhaps conduct epidemiological studies) supporting or refuting a cause-and-effect relationship. This is most important in locations where biosolids are being implicated for disease symptoms.

Principles for establishing causality are described in Hill (1965). These include strength of association, consistency of association (e.g., observation of the symptoms near multiple biosolids application sites), specificity of association, relationships between timing of application and onset of symptoms, biological gradient (dose-response relationship), plausibility of the causative relationship, coherence of evidence,

observation in experiments and analogy to known associations (e.g., occupational exposures to pathogens in biosolids). Hill's principles may be used to determine whether land application of biosolids causes particular diseases. The analysis plan for site-specific risk assessments where disease has been observed might include methods that are not pertinent to national-scale assessments. For example, DNA fingerprinting methods can be used to determine whether pathogens isolated from sick individuals have originated from land-applied biosolids (Dowd and Pillai, 1999; NRC, 2002). Santo Domingo et al. (2007) provide methods to track sources of fecal pollution. Epidemiological studies are discussed below. Risk assessors for site-specific human health assessments might also benefit from guidance for identifying stressors to specific aquatic ecosystems in the *Stressor Identification Guidance Document* (U.S. EPA, 2000) and CADDIS (http://www.epa.gov/caddis/).

5.4.3. Dose-Response Models for Infection

Empirical effects models quantify the relationship between the dose of a microbial agent and frequency of a particular adverse outcome, such as infection, disease, or mortality. These models may assume a minimum infective dose greater than one organism (which for microbial pathogens is supported by little evidence, see below) or a no-threshold continuous dose-response function. These empirical models allow risk assessors to estimate risk at low doses of pathogens. The equations are derived from exposure of humans or animal models to various concentrations of pathogens.

Microbial dose-response models mathematically represent the measure of the dose that yields the probability of a given adverse effect. For microbes, the models are required to be biologically plausible and should consider that a population of humans exposed to infectious microbes will receive a distribution of actual doses (Haas et al., 1999). Also, infectious microbes have the ability to propagate within a susceptible host at an appropriate location within the body (Haas et al., 1999).

Several dose-response models have been used to assess human health risk from microbial agents. These models include exponential dose-response, beta-Poisson dose-response and simple and variable threshold models. These models have been used to assess risk from waterborne and food-borne exposures to microbial agents and recently in risk assessments of pathogens in dewatered, land-applied biosolids (Dowd et al., 2000; Brooks et al., 2005b; Eisenberg et al., 2004). Table 9 provides examples of dose-response models for microbial agents that may be associated with biosolids. Almost all of these examples pertain to the endpoint of infection rather than disease. Further reading and examples of critically analyzed dose-response curves for microbial agents that may be associated with biosolids are presented in Chapter 9 of *Quantitative Microbial Risk Assessment* (Haas et al., 1999).

Infective doses reported for various bacteria, viruses, and protozoan and helminth parasites are tabulated in Epstein (2006) and Gutierrez (2005). However, Haas et al. (1999) argue that most evidence supports the independent action (or single-organism) hypothesis that even a single organism can initiate an infection. Risk assessors might view reported infective doses as doses where infection becomes likely rather than actual thresholds.

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TABLE 9

Examples of Dose-Response Models for Microbial Agents

Organism	Measure of Exposure	Model	Endpoint	Reference
Rotavirus	Dose	Exponential Beta-Poisson Log-probit	Human Infection	Ward et al. (1986), Haas et al. (1999)
Cryptosporidium parvum	Dose	Exponential	Human Infection	Dupont et al. (1995)
Cryptosporidium parvum	Dose	Beta-Poisson	Human Infection	Englehardt and Swartout (2004)
Cryptosporidium parvum	Dose	Beta-Poisson	Gastroenteric illness	Englehardt and Swartout (2006)
Enteric virus	Dose	Beta-Poisson	Human Infection	Gerba et al. (2002)
Salmonella serovar Anatum	Dose	Beta-Poisson	Human Infection	McCullough and Eisele (1951), Haas et al. (1999)
Coxsackievirus B3	Dose	Exponential	Human Infection	Dowd et al. (2000)
Salmonella serovar Typhi	Dose	Beta-Poisson	Human Infection	Dowd et al. (2000)
E.coli (0111)	Dose	Beta-Poisson	Human Infection	Ferguson and June (1952), Haas et al. (1999)
E. coli (055)	Dose	Beta-Poisson	Human Infection	June et al. (1953), Haas et al. (1999)
Endotoxin	Concentration in air	Threshold	Decreased lung efficiency, Organic Toxic Dust Syndrome	Baker et al. (1986)

Dose-response models represent major information gaps for risk assessments related to pathogens in biosolids. Most dose-response models have been developed from human or animal feeding studies or from investigations of outbreaks caused by contaminated food without apparent biosolids involvement (Haas et al., 1999). Dose-response relationships are not available for all of the pathogens potentially found in biosolids (see stressor characterization chapter). Dose-response relationships are not available for inhaled microorganisms (NRC, 2002). As stated in the literature review (Appendix A), the percentage of inhaled pathogens that are ingested is unknown. Dose-response models are also not available for dermal exposure. Furthermore, few dose-response models are available for disease.

5.4.4. Predicting Disease

Existing risk assessment studies for pathogens in biosolids estimate risk of human infection rather than risk of disease (see literature review in Appendix A). If limited by existing data, risk assessments for diseases caused by pathogens in biosolids would be highly uncertain.

Disease is a function of a “triad,” the interaction of pathogen, host and environment. All three factors figure into assessing the incidence of disease in individuals, and the problem formulation should include a plan for analysis of all three aspects. The pathogen is the causative agent of the disease. Whereas chemicals are generally assumed to elicit comparable responses in appropriate animal models as do humans, pathogens are more host-specific. Pathogens can elicit adverse responses

either through their own biological activity within the host or through the production of toxic byproducts.

The second aspect of disease is the host condition. The disease manifestation can vary considerably among infected individuals based on nutritional and health status, and immune profile. Individuals in good health with a history of prior exposure to similar strains of pathogens are less likely to exhibit pronounced symptoms than individuals in poor health or without prior exposure. Immunity is one of the most important parameters influencing the risk from pathogens in biosolids, based on Eisenberg et al.'s (2004) model. The analysis plan should specify whether groups of individuals of particular immune status are assessment endpoint entities in the risk assessment. However, validated protocols are not available to incorporate immune status or other pathogen susceptibility factors (pregnancy, age) into risk assessments (NRC, 2002).

The environment aspect of the triad refers to conditions which promote or retard the ability of the organism to survive in various media and which contribute or limit the spread of the organisms to a receptor. For the most part, the environment is addressed in the exposure components of the conceptual model and is pertinent to infection rather than disease. An assessment of disease incidence cannot proceed without an understanding of these factors and how they influence individual components of the model.

5.4.4.1. *Risk Assessment Model*

Eisenberg et al. (2004, 2005, 2006) developed a methodology to assess risks to human health from pathogens in biosolids-amended soil. While many of the processes in the model are those described in this chapter (fate, transport, uptake), others may not

be needed. For example, Eisenberg et al. modeled the attenuation of organisms in sewage sludge, but it is just as easy to measure concentrations in biosolids as in sewage sludge. Thus, that component of their model is unnecessary. Eisenberg et al. also modeled secondary transmission, which is important for estimating the total burden of disease. However, secondary transmission of pathogens is not unique to the biosolids context, and it is not discussed in this problem formulation, which is concerned with risks of primary infection.

5.4.4.2. *Role of Epidemiology*

Epidemiological assessments of land-applied biosolids would provide much needed information concerning the potential for adverse impact to human health following land application of biosolids. Presently, few data exist to provide insight as to whether a causative association exists between applied biosolids and adverse health effects. Temporal and spatial relationships between time of application and onset of symptoms or other indicators would identify key routes of exposure to assess the validity of the conceptual models presented here and to prioritize exposure scenarios. Epidemiological assessments would focus on studies or disease reports (clustering of illness cases) that can draw a link between those individuals living in close proximity to sites of application and members of farm families and workers who apply biosolids to determine if those individuals have a higher incidence of disease over time.

Risk assessments which use epidemiological studies of sites on or near places of biosolids application would be based on the collection of several key data. First, the data should indicate whether individuals living on or near lands receiving biosolids have a higher incidence of infection compared with cohorts at more distant locations.

Second, data should identify temporal relationships between time and duration of application and onset of symptoms. Such relationships could indicate potential route of exposure—rapid onset may suggest aerosol exposure, whereas delayed disease may indicate an alternate exposure route. Third, data should establish a concordance of symptoms which could also help to determine the route of exposure and whether a single or multiple pathogens are responsible for the effects. Collectively, this information will help to determine if there is a significant microbial risk associated with the use of Class B biosolids and, if so, to help to refine conceptual models and to identify the primary data and methods needed for the risk assessment.

Additionally, epidemiological information for biosolids amendments should focus on plausible exposure scenarios and the characterization of potentially exposed cohorts. First, identifying the exposure settings provides a link between biosolids application and environmental transport of pathogens and exposure points for human contact. Second, data on potentially exposed populations should be identified using information on proximity to the site of biosolids application, climatic conditions and temporal relationships between posited exposures and the onset of infection or clinical symptoms. The selection of appropriate cohorts is important along with the availability of supporting medical information, such as isolates of pathogens and/or serology demonstrating infection within a time frame that corresponds with a plausible exposure scenario (e.g., time of application, environmental transport, exposure point, exposure route, infection, etc.).

Risk assessors should be aware of the difficulties in conducting an epidemiological study of biosolids exposure. In theory, it is unlikely that land application

of properly treated Class B biosolids would result in adverse health impacts. Few people who are exposed are expected to become infected, and even fewer to manifest symptoms of disease. Also, various symptoms may be associated with one pathogen, and various pathogens can cause similar symptoms (Simmonds, 2005). However, a recent conference abstract indicates that an epidemiological study of biosolids exposure is underway (Heaney et al., 2007).

5.5. PLAN FOR RISK CHARACTERIZATION

The analysis plan should include a plan for conducting the risk characterization, which is the phase of risk assessment that integrates the characterization of exposure and the exposure-response relationships to estimate the likelihood of health effects endpoints.

5.5.1. Screening Risk Assessment

The analysis plan must describe whether the risk assessment will include a screening-level risk characterization to eliminate pathways, pathogens, or scenarios that are clearly not of concern. A screening analysis typically makes use of effects standards or benchmarks, but pathogen levels in biosolids that would result in a very low and acceptable dosage of pathogens are not available. Screening analysis can also eliminate pathways using qualitative information (e.g., obvious lack of contact between pathogens and residents in an area devoid of residences). A risk assessor with sufficient resources could develop critical distances for potential risk associated with the bioaerosol transport pathway, and thus eliminate scenarios where there are no people within the critical distance. Screening analysis is usually conducted for

information-rich risk assessment topics, which risk assessments for pathogens in biosolids are not expected to be.

5.5.2. Weight of Evidence

If multiple lines of evidence are expected, the analysis plan should explain how these results will be weighed. For example, an unvalidated animal model might predict a certain infection rate, but epidemiological evidence might show that the only disease outbreak was probably associated with a local crop to which biosolids was not applied. In this case, the latter evidence might be given a higher weight. Each line of evidence links an exposure estimate with an effects estimate, and qualitative or quantitative weights may be given to the combined risk estimate. Evidence from measures of pathogen levels in aerosols might be weighted more than evidence from modeled estimates based on measures of biosolids-amended soils. Evidence from well designed epidemiological studies might be weighted more than evidence from rodent studies that have not been corroborated with epidemiological evidence. Suter et al. (2000) provide criteria for weighing evidence: relevance to the assessment endpoint, demonstrated relationship between exposure and response, temporal scope of evidence compared to temporal variance, spatial scope of evidence compared to spatial area of interest, data quality, number of observations and uncertainty of evidence. Given the paucity of exposure and effects data for risk assessments of land-applied biosolids, weight-of-evidence procedures may be infrequent.

5.5.3. Uncertainty Analysis

Uncertainty analysis is the component of the risk characterization that reveals the uncertainties of the exposure or risk estimate in quantitative or qualitative terms. The management goal of uncertainty analysis may be simply to describe uncertainties, to rank uncertainties or to calculate a probabilistic endpoint. In the case of pathogens in biosolids, probabilistic endpoints might be generated from variability and uncertainty in measurements of pathogens in biosolids, outputs of transport models or outputs of dose-response models. Haas et al. (1999) divided uncertainty into parameter uncertainty, which is related to measurement, and model uncertainty, which is related to the structure of the equations (e.g., whether an important factor was missing from the model). The uncertainties associated with the sampling and modeling methods are described above in the relevant sections. When new data are needed and cannot be obtained, risk pathways that cannot be assessed are a source of uncertainty and should be described in the analysis plan. Risk assessors need to distinguish between pathways that are unquantifiable and pathways that are deemed negligible based on evidence.

6. REFERENCES

Baker, J., S. Curtis, O. Hogsett et al. 1986. Safety in swine production systems. In: Pork Industry Handbook. PIH-104 Cooperative Extension Service, Purdue University, West Lafayette, IN (as cited in Smith et al., 2005a).

Barbier, D., D. Perrine, C. Duhamel, R. Doublet and P. Georges. 1990. Parasitic hazard with sewage sludge applied to land. Appl. Environ. Microbiol. 56:1420-1422.

Batie, S.S. 1983. Soil Erosion: Crisis in America's Croplands? The Conservation Foundation, Washington, DC.

Bowman, D.D. and R. Fayer. 2005. Concerns related to protozoan and helminth parasites in biosolids and animal wastes. In: Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure, J.E. Smith, Jr., P. Millner, W. Jakubowski, N. Goldstein and R. Rynk, Eds. The J.G. Press, Inc., Emmaus, PA. p. 127-162 Compost Science and Utilization.

Bowman, D.D., R.S. Riemers, M.D. Little, M.B. Jenkins, W.S. Bankston and M.M. Atique. 2000. Assessment and comparison of Ascaris egg and Cryptosporidium oocyst inactivation with respect to biosolids processing. In: 14th Annual Residuals and Biosolids Management Conference Water Environment Federation, Alexandra, VA.

Brady, N.C. 1974. The Nature and Properties of Soils. Macmillan Publishing, Co., New York, NY.

Brooks, J.P., B.D. Tanner, K.L. Josephson, C.P. Gerba and I.L. Pepper. 2004. Bioaerosols from the land application of biosolids in the desert southwest USA. Water Sci. Technol. 50:7-12.

Brooks, J.P., B.D. Tanner, C.P. Gerba, C.N. Haas and I.L. Pepper. 2005a. Estimation of bioaerosol risk of infection to residents adjacent to a land applied biosolids site using an empirically derived transport model. J. Appl. Microbiol. 98:397-405.

Brooks, J.P., B.D. Tanner, K.L. Josephson, C.P. Gerba, C.N. Haas and I.L. Pepper. 2005b. A national study on the residential impact of biological aerosols from the land application of biosolids. J. Appl. Microbiol. 99:310-322.

Brooks, J.P., B.D. Tanner, C.P. Gerba and I.L. Pepper. 2006. The measurement of aerosolized endotoxin from land application of Class B biosolids in Southeast Arizona. Can. J. Microbiol. 52:150-156 (as cited in Pepper et al., 2006).

Brooks, J.P., S.L. Maxwell, C. Rensing, C.P. Gerba and I.L. Pepper. 2007. Occurrence of antibiotic-resistant bacteria and endotoxin associated with the land application of biosolids. Can. J. Microbiol. 53:616-622.

Brown, L.M. 2000. Helicobacter pylori: Epidemiology and routes of transmission. Epidemiol. Rev. 22:283-297.

Bujoczek, G., R.S. Reiners and J.A. Olaszkiewicz. 2001. Abiotic factors affecting inactivation of pathogens in sludge. Water Sci. Technol. 44:79-84.

CDC (Centers for Disease Control and Prevention). 1999. Prevention of hepatitis A through active or passive immunization: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm. Rep. 48(RR-12):1-37.

Chapron, C.D., N.A. Ballester and A.B. Margolin. 2000. The detection of astrovirus in sludge biosolids using an integrated cell culture nested PCR technique. J. Appl. Microbiol. 89:11-15.

Chetochine, A.S., M.L. Brusseau, C.P. Gerba and I.L. Pepper. 2006. Leaching of phage from Class B biosolids and potential transport through soil. Appl. Environ. Microbiol. 72:665-671.

Clark, C.S., R. Rylander and L. Larsson. 1983. Levels of gram-negative bacteria, *Aspergillus fumigatus*, dust and endotoxin at compost plants. Appl. Environ. Microbiol. 72:665-671.

Dahab, M.F. and R.Y. Surampalli. 2002. Effects of aerobic and anaerobic digestion systems on pathogen and pathogen indicator reduction in municipal sludge. Water Sci. Technol. 46:181-187.

DeLuca, G., F. Zanetti, P. Fateh-Moghadm and S. Stampi. 1998. Occurrence of *Listeria monocytogenes* in sewage sludge. Zentralbl Hyg Umweeltmed. 201:269-277 (as cited in Pepper et al., 2006).

DeLuzio, N.R. and T.J. Friedman. 1973. Bacterial endotoxins in the environment. Nature. 224:49-51.

Dorn, C.R., C.S. Reddy, D.N. Lamphere, J.V. Gaeuman and R. Lanese. 1985. Municipal sewage sludge application on Ohio farms: Health effects. Environ. Res. 38:332-359.

Dowd, S.E. and S.D. Pillai. 1999. Identifying the sources of biosolid derived pathogen indicator organisms in aerosols by ribosomal DNA fingerprinting. J. Environ. Sci. Health A. 34:1061-1074.

Dowd, S.E., K.W. Widmer and S.D. Pillai. 1997. Thermotolerant Clostridia as an airborne pathogen indicator during land application of biosolids. J. Environ. Qual. 26:194-199.

Dowd, S.E., C.P. Gerba, I.L. Pepper and S.D. Pillai. 2000. Bioaerosol transport modeling and risk assessment in relation to biosolid placement. J. Environ. Qual. 29:343-348.

Dupont, H., C. Chappell, C. Sterling, P. Okhuysen, J. Rose and W. Jakubowski. 1995. Infectivity of *Cryptosporidium parvum* in healthy volunteers. N. Engl. J. Med. 332:855-859.

Eisenberg, J.N.S., J.Q. Soller, J. Scott, D.M. Eisenberg and J.M. Colford, Jr. 2004. A dynamic model to assess microbial health risks associated with beneficial uses of biosolids. Risk Anal. 24:221-236.

Eisenberg, J.N.S., J.A. Soller, J. Scott, D.M. Eisenberg and J.M. Colford, Jr. 2005. A dynamic model to assess microbial health risks associated with beneficial uses of biosolids. In: Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure, J.E. Smith, Jr., P. Millner, W. Jakubowski, N. Goldstein and R. Rynk, Eds. Compost Science and Utilization/The J.G. Press, Inc., Emmaus, PA. p. 177-194.

Eisenberg, J.N.S., K. Moore, J.M. Colford, Jr., J.A. Soller and D.M. Eisenberg. 2006. Application of a Dynamic Model to Assess Microbial Health Risks Associated with Beneficial Uses of Biosolids. Water Environment Research Foundation, Alexandria, VA. 98-REM-1a.

Englehardt, J. and J. Swartout. 2004. Predictive population dose-response assessment for *Cryptosporidium parvum*: Infection endpoint. J. Toxicol. Environ. Health A 67:651-666.

Englehardt, J.D. and J. Swartout. 2006. Predictive Bayesian microbial dose-response assessment based on suggested self-organization in primary illness response: *Cryptosporidium parvum*. Risk Anal. 26:543-554.

Epstein, E. 2006. Pathogens and pathogenic substances in biosolids and manures. J. Residuals Sci. Technol. 3:71-77.

Epstein, E. and L.H. Moss. 2006. A comparison of characteristics of manures, biosolids, and mineral fertilizers. J. Residuals Sci. Technol. 3:35-42.

Evanylo, G.K. 1999. Agricultural land application of biosolids in Virginia: Managing biosolids for agricultural use. Virginia Cooperative Extension, Virginia Tech, Blacksburg, VA. Publication Number 452-303.

Ferguson, W.W. and R.C. June. 1952. Experiments on feeding adult volunteers with *Escherichia coli* 111 B_4: A coliform organism associated with infant diarrhea. Am. J. Hyg. 55:155-169.

Gale, P. 2003. Using event trees to quantify pathogen levels on root crops from land application of treated sewage sludge. J. Appl. Microbiol. 94:35-47.

Gale, P. 2005a. Pathogens in biosolids -- microbiological risk assessment. In: Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure, J.E. Smith, Jr., P. Millner, W. Jakubowski, N. Goldstein and R. Rynk, Eds. Compost Science and Utilization/The J.G. Press, Inc., Emmaus, PA. p. 177-194.

Gale, P. 2005b. Land application of treated sewage sludge: Quantifying pathogen risks from consumption of crops. J. Appl. Microbiol. 98:380–396.

Gale, P. and G. Stanfield. 2001. Towards a quantitative risk assessment for BSE in sewage sludge. J. Appl. Microbiol. 91:563-569.

Gerba, C.P., J.B. Rose and C.N. Haas. 1996. Sensitive populations: Who is at greatest risk? Intl. J. Food Microbiol. 30:113-123.

Gerba, C.P., I.L. Pepper and L.F. Whitehead, III. 2002. A risk assessment of emerging pathogens of concern in the land application of biosolids. Water Sci. Technol. 46:225-230.

Gerba, C.P. 2005. Enteric viruses in biosolids. In: Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure, J.E. Smith, Jr., P. Millner, W. Jakubowski, N. Goldstein and R. Rynk, Eds. Compost Science and Utilization/The J.G. Press, Inc., Emmaus, PA.

Gerba, C.P. and V. Smith, Jr. 2005. Sources of pathogenic microorganisms and their fate during land application of wastes. J. Environ. Qual. 34:42-48.

Goyal, S.M., S.A. Schaub, F.M. Wellings et al. 1984. Round robin investigation of methods for recovering human enteric viruses from sludge. Appl. Environ. Microbiol. 48:531-538.

Gregersen, P., K. Grunnet, S.A. Uldum, B.H. Andersen and H. Madsen. 1999. Pontiac fever at a sewage treatment plant in the food industry. Scand. J. Work Environ. Health 26:291-295 (as cited in Pepper et al., 2006).

Guntupalli, R., R.S. Lakshmanan, J. Hu et al. 2007. Rapid and sensitive magnetoelastic biosensors for the detection of *Salmonella typhimurium* in a mixed microbial population. J. Microbiol. Meth. 70:112-118.

Gutierrez, S. 2005. Why a workshop on emerging infectious disease agents and issues associated with animal manures, biosolids, and other similar by-products? In: Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure, J.E. Smith, Jr., P. Millner, W. Jakubowski, N. Goldstein and R. Rynk, Eds. Compost Science and Utilization/The J.G. Press, Inc., Emmaus, PA. p. 25-31.

Haas, C.N., J.B. Rose and C.P. Gerba. 1999. Quantitative microbial risk assessment. John Wiley and Sons, Inc., New York, NY (as cited in Brooks et al., 2005).

Heaney, C., W. Steven and A. Lowman. 2007. Study design features of a longitudinal investigation of acute health effects and community-level class B biosolids exposure: Subject recruitment and exposure assessment. Epidemiology. 17(6)(Suppl. S):S443-S443.

Hill, A.B. 1965. The environment and disease: Association or causation? Proc. R. Soc. Med. 58:295-300.

ILSI (International Life Sciences Institute). 2000. Revised Framework for Microbial Risk Assessment. ILSI Risk Science Institute, Washington, DC. Available at http://rsi.ilsi.org/file/mrabook/pdf.

Jacques Whitford Limited. 2004. Biosolids Pellet Review Study. Human Health and Ecological Risk Assessment. Submitted to Toronto Public Health, Toronto, Ontario, Canada. Available at http://www.toronto.ca/health/hphe/pdf/abtp_presentation2.pdf.

June, R.C., W.W. Ferguson and M.T. Worfel. 1953. Experiments in feeding adult volunteers with *Escherichia coli* 55 B_5: A coliform organism associated with infant diarrhea. Am. J. Hyg. 57:222-236.

Lang, N.L., S.R. Smith, D.M. Bellett-Travers, E.B. Pike and C.L. Rowlands. 2003. Decay of *Escherichia coli* in soil following the application of biosolids to agricultural land. Water Environ. Manage. J. 17:23-28.

Lewis, D.L. and D.K. Gattie. 2002. Pathogen risks from applying sewage sludge to land. Environ. Sci. Technol. 36:287a-293a.

Lewis, D.L., D.K. Gattie, M.E. Novak, S. Sanchez and C. Pumphrey. 2002. Interactions of pathogens and irritant chemicals in land-applied sewage sludges (biosolids). BMC Pub. Health. 2:11. Available at http://www.biomedcentral.com/1471-2458/2/11.

Lubick, N. 2007. Estimating aerosolized contaminants from class B biosolids. Environmental Science and Technology Online News. Available at http://pubs.acs.org/subscribe/journals/esthag-w/2007/apr/science/nl_biosolids.html (accessed April 4, 2007).

Lytle, D.A., E.W. Rice, C.H. Johnson and K.R. Fox. 1999. Electrophoretic mobilities of *Escherichia coli* O157:H7 and wild-type *Escherichia coli* strains. Appl. Environ. Microbiol. 65:3222-3225.

McCullough, N.B. and C.W. Eisele. 1951. Experimental human salmonellosis. I. Pathogenicity of strains of *Salmonella meleagridis* and *Salmonella anatum* obtained from spray dried whole egg. J. Infec. Dis. 88:278-289.

McFarland, M.J. 2000. Biosolids Engineering. McGraw Hill, New York, NY.

Monroe, S.S., T. Ando and R.I. Glass. 2000. Introduction: Human ecteric caliciviruses—an emerging pathogen whose time has come. J. Infect. Dis. 181(Suppl. 2):S249-S251.

Montemagno, C.D., L.L. Yeghiazarian and P. Kalita. 2004. Field Calibration and Verification of a Pathogen Transport Model. Water Environment Research Foundation, Washington, DC. 00-WSM-3. Available at http://www.werf.org/AM/Template.cfm?Section=Preparing_a_Report&Template=/CM/ContentDisplay.cfm&ContentFileID=22.

Nappier, S.P., M.D. Aitken and M.D. Sobsey. 2006. Male-specific coliphages as indicators of thermal inactivation of pathogens in biosolids. Appl. Environ. Microbiol. 72:2471-2475.

NRC (National Research Council). 2002. Biosolids applied to land: Advancing standards and practices. National Academy Press, Washington, DC.

Nwachuku, N. and C.P. Gerba. 2004. Microbial risk assessment: Don't forget the children. Curr. Opin. Biotechnol. 7:206-209.

Paez-Rubio, T. and J. Peccia. 2005. Estimating solar and nonsolar inactivation rates of airborne bacteria. J. Environ. Eng. 131:612-617.

Paez-Rubio, T., X. Hua, J. Anderson and J. Peccia. 2006. Particulate matter composition and emission rates from the disk incorporation of class B biosolids into soil. Atmos. Environ. 40:7034-7045.

Paez-Rubio, T., A. Ramarui, J. Sommer, H. Xin, J. Anderson and J. Peccia. 2007. Emission rates and characterization of aerosols produced during the spreading of dewatered Class B biosolids. Environ. Sci. Technol. 41:3537-3544.

Peccia, J., H.M. Werth, S. Miller and M. Hernandez. 2001. Effects of relative humidity on the ultraviolet induced inactivation of airborne bacteria. Aerosol Sci. Technol. 35:728-740 (as cited in Pillai and Ricke, 2002).

Pepper, I.L., J.P. Brooks and C.P. Gerba. 2006. Pathogens in biosolids. Adv. Agron. 90:1-41.

Pillai, S.D. 2007. Bioaerosols from land applied biosolids: Issues and needs. Water Environ. Res. 79:270-278.

Pillai, S.D. and S.C. Ricke. 2002. Bioaerosols from municipal and animal wastes: Background and contemporary issues. Can. J. Microbiol. 48:681-696.

Pillai, S.D., K.W. Widmer, S.E. Dowd and S.C. Ricke. 1996. Occurrence of airborne bacteria and pathogen indicators during land application of sewage sludge. Appl. Environ. Microbiol. 62:296-299.

Rusin, P.A., S.L. Maxwell, J.P. Brooks, C.P. Gerba and I.L. Pepper. 2003a. Evidence for the absence of *Staphylococcus aureus* in land applied biosolids. Environ. Sci. Technol. 37:4027-4030.

Rusin, P.A., S.L. Maxwell, J.P. Brooks, C.P. Gerba and I.L. Pepper. 2003b. Response to comment on "Evidence for the absence of *Staphylococcus aureus* in land applied biosolids". Environ. Sci. Technol. 37:5836.

Santo Domingo, J.W., D.G. Bambie, T.A. Edge and S. Wuertz. 2007. Quo vadis source tracking? Towards a strategic framework for environmental monitoring of fecal pollution. Water Res. 41:3539-3552.

Simmonds, C. 2005. Pathogens in biosolids: Risks and regulations. In: Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure, J.E. Smith, Jr., P. Millner, W. Jakubowski, N. Goldstein and R. Rynk, Eds. Compost Science and Utilization/The J.G. Press, Inc., Emmaus, PA. p. 231-238.

Smith, J.E., P D. Millner and N. Goldstein. 2005a. Highlights, insights, and perspectives on infectious disease agents in sewage sludge and animal manure in the United States. In: Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure, J.E. Smith, Jr., P. Millner, W. Jakubowski, N. Goldstein and R. Rynk, Eds. Compost Science and Utilization/The J.G. Press, Inc., Emmaus, PA. p. 3-23.

Smith, J.E., Jr., P. Millner, W. Jakubowski, N. Goldstein and R. Rynk. 2005b. Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure. Compost Science and Utilization/The J.G. Press, Inc., Emmaus, PA.

Soller, J.A., J.N.S. Eisenberg, D.M. Eisenberg and J. Colford. 2006. Research Digest: Use of Risk Assessment to Evaluate Human Health Risks Associated with Pathogens in Biosolids. Water Environment Research Foundation, Alexandria, VA. 98-REM-1b.

Spicer, R.C. and J.J. Gangloff. 2000. Limitations in application of Spearman's rank correlation to bioaerosols sampling data. Am. Ind. Hyg. Assoc. J. 61:362-366.

Stine, S.W., I. Song, C.Y. Choi and C.P. Gerba. 2005. Effect of relative humidity on preharvest survival of bacterial and viral pathogens on the surface of cantaloupe, lettuce, and bell peppers. J. Food Protect. 68:1352-1358.

Straub, T.M., I.L. Pepper and C.P. Gerba. 1993. Hazards from pathogenic microorganisms in land-disposed sewage sludge. Rev. Environ. Contam. Toxicol. 132:55-91.

Suter, G.W., II. 1999. Developing conceptual models for complex ecological risk assessments. Hum. Ecol. Risk Assess. 5:375-396.

Suter, G.W., II, R.A. Efroymson, B.E. Sample and D.S. Jones. 2000. Ecological Risk Assessment for Contaminated Sites. Lewis Publishers/CRC Press, Boca Raton, FL.

Tanner, B.D. 2004. Aerosolization of microorganisms and risk of infection from reuse wastewater residuals. Graduate School of Medicine, The University of Arizona, Tucson, AZ (as cited in Brooks et al., 2005a).

Tanner, B.D., J.P. Brooks, C.N. Haas, C.P. Gerba and I.L. Pepper. 2005. Bioaerosol emission rate and plume characteristics during land application of liquid Class B biosolds. Environ. Sci. Technol. 39:1584-1590.

U.S. DOE (Department of Energy). 2006. Risk Assessment Information System. Office of Environmental Management, Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency). 1992. Technical Support Document for Land Application of Sewage Sludge, Vol. II. Appendices. Eastern Research Group, Lexington, MA. EPA 822/R-93-001b. Prepared for Office of Water, Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency). 1993. The Standards for the Use or Disposal of Sewage Sludge. Final Rules. Federal Register, Washington, DC. EPA 822/Z-93-001.

U.S. EPA (U.S. Environmental Protection Agency). 1994. Guidance for the Data Quality Objectives Process. U.S. Environmental Protection Agency, Quality Assurance Management Staff, Washington, DC. EPA QA/G-4.

U.S. EPA (U.S. Environmental Protection Agency). 1995. A Guide to the Biosolids Risk Assessments for the EPA Part 503 Rule. U.S. Environmental Protection Agency, Office of Wastewater Management, Washington, DC. EPA 832/B-93-005. Available at http://www.epa.gov/owm/bio/503rule/index.htm.

U.S. EPA (U.S. Environmental Protection Agency). 1997. Exposure Factors Handbook, Vol. 1. General Factors. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Washington, DC. EPA/600/P-95/002fa. Available at http://www.epa.gov/ncea/pdfs/efh/front.pdf.

U.S. EPA (U.S. Environmental Protection Agency). 1998. Guidelines for Ecological Risk Assessment. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC. EPA 630/R-95-002f.

U.S. EPA (U.S. Environmental Protection Agency). 1999. Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC. EPA 832/R-92-013. Available at http://www.epa.gov/ttbnrml/625/R-92/013.htm (as cited in NRC, 2002).

U.S. EPA (U.S. Environmental Protection Agency). 2000. Stressor Identification Guidance Document. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC. EPA 822/B-00-025.

U.S. EPA (U.S. Environmental Protection Agency). 2003a. Framework for Cumulative Risk Assessment. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC. EPA/630/P-02/001F.

U.S. EPA (U.S. Environmental Protection Agency). 2003b. Standards for the use or disposal of sewage sludge. In: Final Agency Response to the National Research Council Report on Biosolids Applied to Land and the Results of EPA's Review of Existing Sewage Sludge Regulations. U.S. Environmental Protection Agency, Office of Wastewater Management, Washington, DC.

University of Washington. 2002. Using biosolids for remediation and reclamation of disturbed soils. Center for Urban Horticulture, University of Washington, Seattle, WA.

Virginia Department of Health. 1999. The biosolids lifecycle. Division of Wastewater Engineering.

Ward, R.L., D.L. Bernstein, E.C. Young, J.R. Sherwood, D.R. Knowlton and G.M. Schiff. 1986. Human rotavirus studies in volunteers: Determination of infectious dose and seriological response to infection. J. Infec. Dis. 154:871-880.

Watkins, J. and K.P. Sleath. 1981. Isolation and enumeration of *Listeria monocytogenes* from sewage, sewage sludge, and river water. J. Appl. Bacteriol. 50:1-9.

Wischmeier, W.H. and D. Smith. 1978. Predicting rainfall erosion losses: A guide to conservation planning. In: USDA-ARS Agriculture Handbook No. 537, Washington, DC.

Woodruff, N.P. and F.H. Siddoway. 1965. A wind erosion equation. Soil Sci. Soc. Am. Proc. 29:602-608.

Yanko, W.A. 2005. Bacterial pathogens in biosolids—Emerging issues. In: Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure, J.E. Smith, Jr., P. Millner, W. Jakubowski, N. Goldstein and R. Rynk, Eds. Compost Science and Utilization/The J.G. Press, Inc., Emmaus, PA. p. 35-49.

Zaleski, K.J., K.L. Josephson, C.P. Gerba and I.L. Pepper. 2005a. Survival, growth, and regrowth of enteric indicator and pathogenic bacteria in biosolids, compost, soil, and land applied biosolids. J. Residuals Sci. Technol. 2:49-63.

Zaleski, K.J., K.L. Josephson, C.P. Gerba and I.L. Pepper. 2005b. Potential regrowth and recolonization of Salmonellae and indicators in biosolids and biosolid-amended soil. Appl. Environ. Microbiol. 71:3701-3708.

APPENDIX A
LITERATURE REVIEW

This appendix presents a literature review that summarizes the available information on microbial risks to humans posed by land-applied biosolids. The review is organized in terms of summary points, research and data gaps, relevant aspects of the NRC (2002) recommendations on biosolids, and data and information available for phases of risk assessments (e.g., fate, transport, uptake, infectivity, risk assessment, causal analysis). Although some studies of pathogens in manures may be relevant to biosolids (e.g., models of pathogen transport), investigations of these untreated materials are beyond the scope of this report. This literature review was completed prior to the other chapters in this report.

SUMMARY POINTS

- The range of pathogens that may be present in biosolids is well understood, but the current national distribution of these pathogens, the variation with type of sewage sludge treatment, and analytical methods for detecting and quantifying pathogens are not well understood or developed.
- Many analytical methods for detecting and quantifying pathogens focus on detecting DNA sequences rather than viable cultures.
- The use of indicator organisms to represent pathogens of concern has the potential to introduce large uncertainties into estimates of exposure.
- Risk assessments of pathogens in biosolids have been performed, but the emphasis has been on the use of particular transport models to quantify risks from a few pathogens to individuals at a distance from particular biosolids application sites rather than the process of planning and conducting a national-scale or other broad risk assessment. A formal problem formulation for pathogens in biosolids has not been undertaken.
- Conceptual models for human health risk assessments of pathogens in biosolids that include detailed source descriptions, transport pathways and routes of exposure have not been developed previously.

- A causal association between exposures to biosolids and adverse effects on human health has not been documented.

- Epidemiological studies of biosolids application sites are generally lacking and are problematic to conduct.

- Although the U.S. EPA has standard exposure factors and effects levels relevant to chemicals, some standard exposure factors and effects levels needed for risk assessments of pathogens in biosolids are not available.

- U.S. EPA does not have a standard quantitative microbial risk assessment framework for use in risk assessments of pathogens in biosolids.

- Dose-response relationships used in risk assessments of pathogens in biosolids have been derived from non-biosolids studies, and it is unclear how applicable these relationships are to biosolids, particularly for the inhalation pathway.

- Although the science of biosolids exposure analysis is still under development, studies of effects of pathogens in biosolids are limited.

- Little information is available to support the elimination of exposure scenarios or pathways from consideration at all sites where biosolids have been applied. Information may support the screening of exposure pathways from consideration at particular sites.

- Bioaerosol emissions from biosolids have been studied most rigorously in Arizona; few data exist for other regions.

- Exposure assumptions vary in existing risk assessments for bioaerosols generated from biosolids.

- Existing risk assessment studies of pathogens in biosolids at specific sites estimate risk of infection rather than risk of disease.

Many of the research and monitoring gaps related to human health risk assessments of biosolids are described in key papers and are summarized in Table A-1. These include aspects of problem formulation, exposure assessment and effects assessment.

1

TABLE A-1

Research, Monitoring, Assessment and Modeling Needs Related to Risk Assessment for Land Application of Biosolids

Need	Reference
Stressor Characterization	
New national survey of pathogens in sewage sludge	NRC (2002)
Research on incidence of prions in biosolids	Pepper et al. (2006)
Research to assess utility of additional indicator microoganisms such as *Clostridium perfringens*	NRC (2002)
Research to assess metabolic status of aerosolized pathogens and environmental and biological factors that influence this metabolic state	Pillai and Ricke (2002)
Research to assess potential for pathogen reproduction within bioaerosols	Pillai and Ricke (2002)
New indicators for viruses in biosolids (judged by cited workgroup to be a medium priority)	Virus workgroup in Smith et al. (2005b)
Measures of Exposure (quantifying pathogens)	
Improvement (e.g., analytical specificity, sensitivity, accuracy), standardization, validation of detection methods for bacteria, viruses, protozoan parasites, helminthic parasites in biosolids	Smith et al. (2005a), NRC (2002), U.S. EPA (2003b)
Standardized methods for measuring and characterizing pathogens in bioaerosols	NRC (2002), Pillai (2002)
Molecular, immunological, immuno-magnetic separation and culture (IMSC) techniques for detection of low numbers of pathogens	Smith et al. (2005a)
Standardization and validation of assays for detecting and enumerating waterborne protozoan parasites (*Cryptosporidium*, *Cyclospora*, *Toxoplasma*, *Microsporidia*, *Balantidium*, *Giardia* and *Entamoeba*), fecal coliforms, *Salmonella* spp., enteric viruses and helminth eggs in biosolids matrices	Smith et al. (2005a)

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TABLE A-1 (cont.)

Need	Reference
Measurement of occurrence, survival, fate and transport of cysts of protozoans and worms/nematodes, as well as viruses or surrogates with respect to different treatment and land application scenarios	Smith et al. (2005a)
Evaluation of the usefulness of surrogates and models to determine presence or survival of infectious agents before and after treatment and land application	Smith et al. (2005a)
Measurement of antibiotic resistance determinants in bacteria in biosolids	Smith et al. (2005a)
Measurements of post-treatment pathogen concentrations, confirmation that Class B treatment combined with use restrictions result in below-detection pathogen concentrations	NRC (2002), Gerba (2005)
Creation of matrix of virus concentrations in different types of biosolids, by source of sewage sludge and type of treatment (judged by cited workgroup to be a medium priority)	Virus workgroup in Smith et al. (2005b)
Measures of Exposure (fate and transport)	
Research on the fate and transport of bioaerosols from land application or spray irrigation	Smith et al. (2005a), NRC (2002)
Better bioaerosol dispersion and viability models	Pillai and Ricke (2002)
Improved bioaerosol samplers that are designed not only for bacterial collection, but also for virus and endotoxin collection	Pillai (2007)
Research to assess transport and fate of viruses in land applied biosolids (judged by cited workgroup to be a medium priority)	Virus workgroup in Smith et al. (2005b)
Monitoring of pathogens at various points in the environmental transport process from the biosolids source to the site of exposure	Eisenberg et al. (2004)
Relationships between pathogen survivorship and environmental factors	Eisenberg et al. (2004)

TABLE A-1 (cont.)

Need	Reference
Development of site-specific atmospheric dispersion models (and research supporting parameter development) to identify appropriate bioaerosol sampling locations depending on micrometeorological conditions	Pillai (2007)
Research on effect of harvest and grazing restrictions on pathogen fate and transport	NRC (2002)
Monitoring to assess potential exposures from runoff from land application of biosolids (judged by cited workgroup to be a medium priority)	Parasite workgroup in Smith et al. (2005b)
Research to assess fate of viruses most resistant to temperature and high pH treatment processes, i.e., hepatitis A and adenoviruses	Pepper et al. (2006)
Monitoring to assess potential for regrowth of *E. coli* O157:H7 after treatment processes	Pepper et al. (2006)
Measurement of fate of *Cryptosporidium* oocysts during treatment and after soil amendment in a variety of environments	Pepper et al. (2006)
Relevance of correlations between indicator and endpoint microorganisms in biosolids to relationships in aerosols	Brooks et al. (2005b)
Measures of Exposure (biotic uptake)	
Research to assess adequacy of 30-day waiting period for grazing following land application of Class B biosolids (judged by cited workgroup to be a medium priority)	Virus workgroup in Smith et al. (2005b)
Measures of Exposure (human parameters)	
Research on exposure of workers and off-site residents to biosolids and biosolids components (bioaerosols, dust)	Smith et al. (2005a) Virus workgroup in Smith et al. (2005b)
Information on actual ingestion and inhalation rates, as well as duration of exposure (e.g., percent of inhaled bacteria that are swallowed)	Gerba and Smith (2005), Brooks et al. (2005b)

TABLE A-1 (cont.)

Need	Reference
Determination of route of exposure of humans to aerosolized pathogens	Pillai (2007)
Information on household-level transmission of pathogens	Eisenberg et al. (2004)
Information on human transmission of pathogens (such as non-typhi *Salmonella*) by inhalation of bioaerosols and associated dose-response relationships	Pepper et al. (2006)
Dose-Response Relationships	
Development of relationships between ingested doses and severity and duration of effects, including species and subspecies differences in infectivity	NRC (2002)
Validation of animal-derived dose-response relationships for humans	NRC (2002)
Tests of models used to extrapolate dose-response relationships derived at high doses to low doses	NRC (2002)
Development of relationships between treatment process conditions (time, temperature, pH, chemical doses, holding times), pathogen indicator concentrations and maximum acceptable pathogen concentrations	NRC (2002)
Research on the role of chemical irritants in affecting pathogen-related risks	Lewis et al. (2002)
Research on infectivity of aerosolized microbial pathogens, especially enteric pathogens	Pillai and Ricke (2002), Pillai (2007)
Determination of infective doses for parasites	Parasite workgroup in Smith et al. (2005b)
Research on minimum infective doses (minimum number of infectious units required to cause an infection), especially for immunocompromised individuals	Lewis and Gattie (2002)

TABLE A-1 (cont.)

Need	Reference
Research on how different pathogen strains interact in the development of immunity	Eisenberg et al. (2004)
Risk Assessment	
Quantitative microbial risk assessment methods	NRC (2002)
Sensitivity analyses to determine what critical information is needed to reduce uncertainty in microbial risk assessments	NRC (2002)
Risk assessment of *Ascaris* ova, which requires data on levels of viable ova in biosolids and survival under different environmental conditions (many limits for use of agricultural land after land application of Class B biosolids are determined by survival of Ascaris ova)	Pepper et al. (2006)
Risk assessment on Class B biosolids and vectors (e.g., flies) for virus transmission (judged by cited workgroup to be a high priority)	Virus workgroup in Smith et al. (2005b)
Risk assessment for exposure of public to Class B biosolids, including scenarios where food crops are grown or harvested (judged by cited workgroup to be a high priority)	Virus workgroup in Smith et al. (2005b)
Population-based risk model related to biosolids properties and properties of pathogens from biosolids	Eisenberg et al. (2004)
Research on management alternatives such as riparian buffers	Smith et al. (2005a)
Validation of health risk models using epidemiological studies	Pillai and Ricke (2002), Pillai (2007)
Causal Analysis	
Demonstration of causal association between biosolids exposures and adverse health outcomes	NRC (2002)
Framework for establishing causation in human health investigations, including (1) studies in response to unusual exposures and unusual occurrences of disease, (2) preplanned studies to characterize exposures of workers and communities and (3) epidemiological studies of biosolids use	NRC (2002)

TABLE A-1 (cont.)	
Need	Reference
Epidemiological studies on exposed populations such as those who apply biosolids including farmers and communities near land application sites	NRC (2002), Dowd et al. (2000)
Rapid response investigations of reported health effects potentially resulting from land application of biosolids	U.S. EPA (2003b) from WERF Biosolids Research Summit

NRC RECOMMENDATIONS

The NRC was asked by U.S. EPA to evaluate "technical methods and approaches used to establish the chemical and pathogen standards for biosolids, focusing specifically on human health protection and not ecological or agricultural issues" (NRC, 2002). NRC recognized the need to reduce uncertainty about potential for adverse human health effects from exposure to biosolids (NRC, 2002).

Many of the committee's recommendations are pertinent to a problem formulation for risk assessment of land application of biosolids. The Committee on Toxicants and Pathogens in Biosolids Applied to Land was asked to perform the following pathogen-related tasks:

- "Review the current standards for pathogen elimination in biosolids and their adequacy for protecting public health. Consider (a) whether all appropriate pathogens were considered in establishing the standards; (b) whether enough information on infectious dose and environmental persistence exists to support current control approaches for pathogens; (c) risks from exposure to pathogens found in biosolids; and (d) new approaches for assessing risks to human health from pathogens in biosolids."

- "Explore whether approaches for conducting pathogen risk assessment can be integrated with those for chemical risk assessment. If appropriate, recommend approaches for integrating pathogen and chemical risk assessments."

Biosolids management practices and recent risk assessment methods were reviewed. The committee reviewed evidence of human health responses to biosolids including anecdotal allegations of disease, reviewed risk assessments and technical data used to develop pathogen standards, and examined management practices of the Part 503 rule. Peer-reviewed literature and government reports on human health effects of biosolids and treated wastewater were reviewed and described in a table in

the NRC report, with no attempt to verify other allegations. The committee noted that a cause and effect relationship between biosolids and adverse health effects has not been documented (NRC, 2002) (Table A-1). Overarching recommendations included: (1) supplementing technological approaches with risk assessments to establish regulatory criteria for pathogens in biosolids; (2) conducting a new national survey of pathogens in sewage sludge; and (3) developing a framework for establishing causation in human health investigations, including (a) studies in response to unusual exposures and unusual occurrences of disease, (b) preplanned studies to characterize exposures of workers and communities and (c) epidemiological studies of biosolids use NRC (2002, Table A-1). Furthermore, the committee recommended that U.S. EPA assess the reliability of biosolids treatment processes, monitor compliance with pathogen standards, conduct environmental hazard surveillance, and study human exposure and health.

More specific recommendations of the NRC committee included the use of new indicator organisms, such as *Clostridium perfringens* in regulation of land application of biosolids (Table A-1). Moreover, the committee recommended that site restrictions, buffer zones and holding periods for applications of Class B biosolids be specific to geographic and site-specific conditions that affect fate and transport of pathogens. The committee recommends verification of site restrictions to determine if they meet their intended pathogen levels (Table A-1).

Regarding risk assessment, the committee recommended that a conceptual site model should be used to identify all potential routes of exposure (NRC, 2002). The committee found that it is not yet possible to integrate pathogen risk assessment with

chemical risk assessment, given the data gaps and paucity of risk assessment methods for complex mixtures. Furthermore, they noted that several exposure pathways were not adequately addressed in the 1993 Part 503 pathogen requirements, including the inhalation pathway, the potential for surface-water contamination by runoff, groundwater contamination and secondary transmission of disease (NRC, 2002). In particular, pathogen transport and survival in bioaerosols is highly uncertain (Table A-1). Many of these research, monitoring and assessment gaps are included in Table A-1.

PATHOGENS

Extensive information is available describing pathogens that may be present in Class B biosolids as well as their potential effects. Pathogens include bacteria, enteric viruses, protozoan pathogens, helminths and others. Articles that provide detailed information on these classes of pathogens include Epstein (2006), Epstein and Moss (2006), Pepper et al. (2006), NRC (2002), Straub et al. (1993) and chapters in Smith et al. (2005b). The list of potential pathogens is long, but little information is available to eliminate particular agents. However, researchers contributing to the Smith et al. (2005b) volume selected and provided criteria for selecting the most significant bacterial, viral and parasitic pathogens.

Many of the articles above provide information on indicators of pathogens in biosolids. Dowd et al. (1997) recommend thermotolerant clostridia as indicators of fecal contamination in bioaerosols. Pillai et al. (1996) found that clostridia and H_2S (hydrogen sulfide) producers were better indicators of airborne biosolids-derived material than traditional bacterial indicators (fecal coliforms and fecal streptococci).

The primary information gap related to stressor characterization is recent national-scale data on the distributions of concentrations of pathogens in biosolids, with respect to method of treatment, acceptable analytical methods for detecting and quantifying pathogens and other variables (Table A-1). Epstein and Moss (2006) cite references regarding probable numbers of fecal coliforms and *Salmonella* spp. in Class B biosolids. Dahab and Surampalli (2002) found that existing treatment systems do achieve Class B requirements under the US 503 rule, while Class A may not be easily achieved.

Biosolids experts distinguish between traditional and emerging pathogens, and Gerba et al. (2002) reviewed the latter. A committee of experts convened at the Workshop on Emerging Infectious Disease Agents and Issues associated with Sewage Sludge, Animal Manures and Other Organic By-Products in Cincinnati, OH, June 2001, concluded that emerging pathogens do not exhibit survival or other properties that are very different from those exhibited by traditional pathogens (Smith et al., 2005a). Pepper et al. (2006) reviewed studies of various traditional and emerging pathogens and summarized which have been detected in biosolids and which have not been detected in biosolids or not studied.

One recent study found that biosolids were not a likely source of *Staphylococcus aureus* exposure or infection (Rusin et al., 2003a). Helminths are probably the most persistent of enteric pathogens (Pepper et al., 2006; Straub et al., 1993). Little research on the survival of protozoan parasites (e.g., *Cryptosporidium* species, *Giardia*) in biosolids-amended soil has been conducted.

It is impossible to test biosolids for all possible pathogens (Smith et al., 2005a). Enteric viruses and helminth ova have been selected as indicators of treatment efficacy because they are resistant to treatment and can be quantified (Smith et al., 2005a).

Chapter 4 in Smith et al. (2005b) provides detection/analytical capabilities and recommendations for bacterial pathogens in biosolids.

MEASURES OF EXPOSURE

Numerous factors determine human exposure to pathogens in biosolids. These include health status of contributors, method of treatment, percent solids, friability, exposure to heat and UV. We have not conducted an exhaustive search for articles on factors that influence the fate of pathogens. The review below presents a sampling of articles on the topic.

Detection of Pathogens

The detection of pathogens in environmental samples such as biosolids-amended soil is inefficient. For example, Rusin et al. (2003a) had a recovery efficiency of 8.7% for *Staphylococcus aureus* in Class B biosolids. Organic matter and high bacterial counts reduce recovery fraction for pathogens (Rusin et al., 2003b).

Decay of Pathogens

Lang et al. (2003) studied the decay of *E. coli* in biosolids-amended sandy loam soil and quantified indigenous *E. coli* in control soils in the United Kingdom. Stine et al. (2005) studied survival of bacterial and viral pathogens on the surface of fruit and

vegetable crops, but not in a biosolids matrix. Straub et al. (1993) reviews studies of survival of pathogens in soil and sewage sludge.

Lewis and Gattie (2002) assert that models typically use data from experiments from enteric organisms such as *E. coli* and *Salmonella* to estimate bacterial survival rates. They point out that these microorganisms are short-lived compared to those that form spores or are encapsulated (such as *Mycobacterium* spp.).

Gerba et al. (2002) investigated which emerging pathogens are likeliest to survive Class B biosolids treatments. Literature was reviewed (1) relating pathogen survival to temperature and environmental variables, (2) documenting pathogen occurrence in biosolids and (3) describing dose-response models for pathogens. The study concluded that adenoviruses and hepatitis A were heat resistant viruses and therefore likely to survive long periods in the environment. *Escherichia coli* O157:H7 and *Listeria montocytogenes* are emerging bacterial pathogens that can survive anaerobic digestion and can sometimes regrow following land application of biosolids. In contrast, the protozoan parasites microsporidia and *Cyclospora* would not survive under high temperatures of anaerobic digestion or under conditions of low moisture.

Reactivation and Regrowth of Pathogens

Zaleski et al. (2005a) asked “Does regrowth occur following reintroduction or recolonization of pathogens after land application or during storage under favorable conditions?” The authors note that regrowth of indicator bacteria and *Salmonella* in biosolids has been observed under certain moisture, temperature and substrate conditions, and when indigenous bacteria are low. Moreover, pathogens in biosolids

may be reduced if they are stored at certain moisture and temperature ranges. In biosolids-amended soils, increased moisture may lead to survival and regrowth of bacterial pathogens. In one study the use of concrete-lined beds for storage during desiccation allowed moisture from rainfall to accumulate in the beds, leading to growth of fecal coliforms and salmonellae added from external sources (Zaleski et al., 2005b). Furthermore, survival rates of bacteria are higher in soil of finer textures (Zaleski et al., 2005a).

Aerial Transport of Pathogens

Pathogens have rarely been measured in biosolid aerosols (Table A-1). Pillai and Ricke (2002) reviewed factors controlling bioaerosol transport, as well as bioaerosol sampling methods and culture-based approaches to the detection and characterization of specific components of bioaerosols.

Brooks et al. (2004) measured bioaerosol emissions during land application of Class B biosolids in the region of Tucson, AZ. The objective was to develop empirical models of the fate and transport of bioaerosols. Pathogens and indicator bacteria were only rarely found in aerosolized samples. These included coliforms and coliphages, which were present at high densities in biosolids, and animal viruses, which were not detected in biosolids. *Clostridum perfringens* was detected only in a small fraction of aerosol samples, but these were present under various weather conditions. The authors suggest that only microorganisms in the aqueous phase of biosolids were able to aerosolize; others remained sorbed to the solid phase (Brooks et al., 2004).

In another study, Brooks et al. (2006) measured aerosolized endotoxin concentrations downwind of a single biosolids-amended site. Levels were generally within limits previously proposed in occupational exposure studies, though peak concentrations occasionally exceeded these limits. Levels of endotoxin in aerosolized soil were sometimes above those associated with biosolids amended-soil, calling into question whether biosolids were the primary source of the endotoxin. Additional studies of bioaerosol transport that included a risk assessment component are described in the section on risk assessment.

Tanner et al. (2005) determined bioaerosol emission rates and plume characteristics during spray application of liquid Class B biosolids. They did not detect coliphages or coliform bacteria just downwind of the biosolids application (approximately a 2-m distance away), though bacteria that had been added to groundwater and sprayed were detected. The researchers concluded that the presence of biosolids reduces aerosolization of microorganisms relative to application of inoculated groundwater. Even if bacteria had been present below detection limits, the duration of exposure to any pathogens just downwind of biosolids application would be expected to be brief because of the moving applicator (Tanner et al., 2005).

Paez-Rubio et al. (2006) investigated the content of bioaerosols produced during the disking of biosolids on an application site in Central Arizona. Biosolids source emission factors (number of microorganisms or mass of biotoxins per area) and emission rates (number of microorganisms or mass of biotoxins per time) were measured for total bacteria, culturable heterotrophic bacteria (HPC), total coliforms, sulfite-reducing *Clostridia*, and endotoxin, as well as PM_{10}. The authors presented a

correlation between microbial concentrations emitted during disking and their content in biosolids. Disking was determined to be a "substantial source of biosolids-derived aerosols" and might be of greater potential concern than other application methods. The emission rate during disking of biosolids was greater than rates that had been measured during spreading of dewatered biosolids by side slinger or spraying of liquid biosolids. For example, total coliform emissions during disking were about two times greater than emissions associated with spreading dewatered biosolids and at least two orders of magnitude greater than maximum emission rates reported by Tanner et al. (2005) during spraying of liquid biosolids (Paez-Rubio et al., 2006). The authors provide a framework for reconstructing aerosol concentrations and emission rates.

In a related study, Paez-Rubio et al. (2007) measured bioaerosol emission rates from the spreading of Class B biosolids with a side-slinging applicator in Arizona. Concentrations of pathogens in bioaerosols were reconstructed from concentrations in bulk biosolids and PM_{10}. Aerosol emission rates of several bacterial indicators were correlated with their concentrations in bulk biosolids. Aerosol emission rates of dewatered biosolids were one to two orders of magnitude higher than those reported for liquid biosolids. Diameters of emitted particles suggest that most were inhalable and possibly respirable. The authors assert that their work "move[s] aerosol studies beyond indicator measurements by estimating specific toxic compound or pathogen aerosol concentrations based on more easily obtained PM_{10} measurements and bulk biosolids analysis—where detection limits are much lower due to the large sample size possible." J. Peccia, one of the authors, notes that rates of recovery of pathogens in aerosols that are reported in the literature are currently only about 10% (Lubick, 2007). The authors

acknowledge that the relationship between source emission rates and bulk biosolids concentration that they present is limited to the type of spreader they used (i.e., a "ProTwin Slinger" side discharge spreader, the most common spreader for biosolids of the 20%-30% solids content range).

Leaching to Groundwater

A review of the literature has concluded that few pathogens from biosolids leach to groundwater (Pepper et al., 2006). For example, Chetochine et al. (2006) measured the numbers and leaching potential of coliphage MS-2, specific to *E. coli*, from Class B biosolids. Much of the phage was sorbed to or associated with solid particles. Following serial extraction, less than 8% of the phage initially present in the biosolids leached from biosolids-amended soil. The phage was not appreciably retained in a column containing a sandy porous medium.

Y. Jin, J. Sims and K. Kniel of the University of Delaware were awarded a USDA grant from 2006 to 2009 to study the fate and transport of viruses in biosolids and their potential to contaminate groundwater and foodcrops as a result of land application of biosolids.

Erosion and Surface Runoff

We did not find information on these mechanisms of transport of pathogens in biosolids.

Pathogens on Crops

Studies of pathogens on crops are described in the section on risk assessment. Also, the USDA grant described above that was awarded to Y. Jin, J. Sims and K. Kniel of the University of Delaware includes an investigation of the contamination of crops.

RISK ASSESSMENT

Risk Assessment Process

Risk assessments of pathogens in biosolids have been performed by various investigators, but the emphasis has been on the use of particular transport models to quantify exposure and risk, rather than the process of planning and conducting a broad risk assessment. One recent risk assessment of biosolids application found that the science of assessing risk from environmental exposure to biological agents, as well as acceptable levels is “under development at the present time” (Jacques Whitford Limited, 2004). Therefore, the focus of that study was altered from the quantification of risk to the effectiveness of a pelletization process to destroy biological agents of potential concern.

Soller et al. (2006) described general methods for conducting health risk assessments of pathogens in biosolids that were developed as part of a Water Environment Research Foundation project. The methods included characteristics of an infectious disease process, including the consideration of multiple transmission pathways and the presence of immunity. Soller et al.’s framework for evaluating human risks associated with microbes in biosolids included an exposure characterization component (quantifying pathogen levels in the environment) and a health effects

component. A schematic diagram displayed several Class A and Class B sludge treatment processes as well as environmental variables affecting exposure (time, temperature and moisture). They described the tradeoff between site-specific monitoring data and more general data on treatment effectiveness and fate and transport of pathogens from points earlier in the waste stream. A conceptual health effects model was also included in the report. This model, first published in Eisenberg et al. (2004), contained six epidemiological states: (1) susceptible state, (2) exposed state (asymptomatic and infectious), (3) carrier state 1 (asymptomatic but infectious, (4) diseased state, (5) carrier state 2 (previously symptomatic, now asymptomatic and infectious) and (6) protected state (postinfectious and noninfectious and some level of immunity). Soller et al. (2006) also included a table of data required to parameterize a basic health effects model.

Although Soller et al. (2006) included information and diagrams useful for developing a problem formulation for pathogens in biosolids, they did not organize it as a problem formulation. These elements are found in the *Guidelines for Ecological Risk Assessment* (U.S. EPA, 1998).

The International Life Sciences Institute (ILSI) developed a framework for microbial risk assessment related to human exposures to waterborne pathogens (ILSI, 2000). The framework describes the stages of risk assessment, including problem formulation, but without providing or citing scientific advice regarding particular pathogens or exposure pathways.

Bioaerosol Pathways

One of the primary research needs identified by the NRC was human exposure to pathogens in bioaerosols (NRC, 2002). Researchers at the University of Arizona conducted a major study to help understand community and worker risk of infection from bioaerosols, as well as to develop methods for modeling transport of pathogens and human exposure (Brooks et al., 2004, 2005a,b, 2006). Prior to that study, the same group of researchers studied bioaerosols in West Texas (Dowd et al., 2000). Conclusions were that community risks were relatively negligible, with worker risks somewhat higher.

Dowd et al. (2000) sampled bioaerosols emitted from anaerobically digested, dewatered biosolids applied in west Texas. The study generated bacterial and virus release rates from large biosolids piles where they were stored prior to application and fields where biosolids were sprayed. Levels of *Salmonella* and an indicator virus (coliphage) were measured. The ratio between the concentration of indicator virus in aerosols and the concentration in biosolids was used to estimate a value for airborne enteric virus (Coxsackievirus). Microbial transport models (a point source model and an aerial source model) were used to generate downwind concentrations. Dose-response models were used to estimate risk to workers on site and nearby residents at least 10 km away. The pathway was assumed to consist of inhalation and swallowing of the pathogen. The single hit exponential model [$p = 1 – \exp(-rN)$] was used to describe the probability of infection by Coxsackievirus B3, and the Beta-distribution model ($p = 1 – [1 + (N/\beta)(2^{1/\alpha}-1)]^{-\alpha}$) was used to describe the risk of infection by *Salmonella* serovar Typhi, where p = probability of infection, N = number of organisms inhaled, β is the ID_{50}, and α

and *r* are parameters that describe the dose-response curve. Under one of the wind speeds in the study (2 m/s), the risk of bacterial and viral infection of workers exposed for one hour at a distance of 100 m is 2E-2 and 3E-2, respectively. Under these conditions, residents at 10 km from the biosolids source were found to be at no risk from aerosolized viruses and low risk of infection from bacteria (2E-4). Under some more moderate and high wind conditions, especially where exposures were for 8 hours or more at distances of 500 m or less from the source, risks of infection of workers (or others) from bioaerosols were close to 1.0. The authors indicated that several sources of conservatism must be considered when evaluating these risk estimates (e.g., the wind does not always come from the same direction, Dowd et al., 2000). Citing comments by Brooks et al. (2004) on the improved efficiency of modern wastewater treatment plants, Pepper et al. (2006) argue that a more realistic estimate of infectivity is five orders of magnitude lower than Dowd's worst case estimates.

Brooks et al. (2005b) undertook a study to estimate risks of microbial infection of residents near biosolids application sites. At 10 sites throughout the U.S. that were amended with either liquid or solid Class B biosolids (five sites in Arizona, two in Washington state, one in Virginia, one in Texas and one in Illinois), they measured HPC bacteria, total coliform bacteria, *E. coli*, *Clostridium perfringens*, coliphage, enteroviruses, hepatitis A virus and noravirus in aerosol samples downwind from application sites. The study distinguished between loading, unloading, land application and background operations. In general, risks of infection were determined to be low, with the greatest risk of infection, 4×10^{-4}, from coxsackievirus A21 released during loading operations.

Brooks et al. (2005b) cited a dissertation of Tanner (2004) in reporting that the risk of infection to a biosolids handler can reach as high as 34% annually from exposure to coxsackievirus A21 and 2% annually from exposure to *Salmonella* species. This study assumed exposure on a daily basis (250 days per year).

Brooks et al. (2005a) developed an empirical transport model for viruses aerosolized during land application of liquid biosolids. Data were generated from collections of bioaerosols in field tests with coliphage MS-2 added to water and sprayed with a biosolids spray application truck. Risks of infection for residents adjacent to land application sites were also calculated at 10^{-7} (realistic) to 10^{-5}. Conservative annual risks were calculated at no more than seven times that value. A second goal of the study was to develop a transport model for bacteria, but *E. coli* used in the study did not typically survive the aerosolization process.

Based on Brooks' studies, Pepper et al. (2006) concludes that overall community risk of infection from bioaerosols during land application was relatively negligible. Occupational risk during land application were higher than community risks but were still low (Brooks et al., 2004). Pillai (2007) cautions against extrapolating these results to different source materials, regions or even parts of a region. Pathogens in biosolids might be more desiccated or inactivated from exposure to ultraviolet light than in other parts of the country.

In a study of bioaerosol emission rates from the spreading of Class B biosolids in Arizona, measured source endotoxin concentrations were greater than reported conservative thresholds for mucous membrane irritation, and most exceeded the threshold for acute bronchial constriction (Paez-Rubio et al., 2007).

Groundwater Pathways

Based on a review of the literature such as Chetochine et al. (2006, above), Pepper et al. (2006) conclude that groundwater contamination from land-applied biosolids is not likely, and therefore human health risks are likely negligible. By extension, pathways by which pathogens in groundwater may contaminate land or surface water via springs or other interactions are also unlikely to be significant for pathogens from biosolids.

Ingestion of Soil

Gerba et al. (2002) used a beta-Poisson model from Haas et al. (1999, $P = 1 - [1 + N/\beta-\alpha]$) to assess the risk of infection and illness from enteric viruses following land application of Class B biosolids, assuming that exposure was from ingestion of biosolids-amended soil. They focused on rotavirus and echovirus 12. Gerba et al. (2002) determined that direct ingestion of biosolids, if they were spread across the surface of the soil, would result in an annual risk from a one time exposure exceeding 1×10^{-4}. They assumed no natural attenuation of virus. Injection of biosolids into the soil results in a risk below this level.

Consumption of Vegetation

Most of the information on risks from the crop ingestion pathway is from the United Kingdom. Consumption of root crops is assumed to represent the worst case scenario because they contain higher proportions of soil than leafy crops and they are often consumed uncooked (Gale, 2005a). Gale (2003) estimated the exposure of root

crops to *Cryptosporidium* and *Salmonella* species from biosolids applied to agricultural land in accordance with the United Kingdom's Safe Sludge Matrix. An approach using event trees combined with empirical data was used to estimate pathogen levels in raw sewage sludge, in treated sludge and biosolids mixed with topsoil and root crops. Expert opinion suggested that up to 2% of root crops by weight may be soil at the point of harvest. Monte Carlo simulations were performed to model variation in salmonella levels on root crops, assuming a Poisson-log-normal distribution of bacterial counts.

Gale (2005b) conducted risk assessments to estimate the number of humans in the United Kingdom at risk from consumption of root crops obtained from areas where biosolids were applied according to the Safe Sludge Matrix regulations. (Gale [2005a] presents a subset of that study.) Seven classes of pathogens were the focus of the study: salmonellas, *Listeria monocytogenes*, campylobacters, *Escherichia coli* O157, *Cryptosporidium parvum*, *Giardia* and enteroviruses. The study showed that if linear decay were assumed to occur and if the treatment process (mesophilic anaerobic digestion or MAD) were assumed to be 100% efficient, potential risks from the seven classes of pathogens were essentially eliminated. If pathogen decay in treated soil was assumed not to occur, then 50 *Giardia* infections were expected in the United Kingdom and less than one infection per year resulting from the other six pathogens. Also if the MAD process was 99% or lower, substantially more infections from *Giardia* and possibly *E. coli* O157 were predicted.

Gale and Stanfield (2001) calculated risks to humans from consumption of vegetable crops contaminated with the bovine spongiform encephalopathy agent in

sewage sludge in the United Kingdom. Pepper et al. (2006) identified the incidence of prions in biosolids as a research priority in the U.S. (Table A-1).

Proliferation of Antibiotic Resistance

In addition to risks to human health from specific pathogens, another relevant indirect health issue is the possible proliferation of antibiotic resistant bacteria. The potential risk is that human pathogenic strains become resistant to overused antibiotics, which can no longer treat the pathogen. Pepper et al. (2006) ask the question "Can antibiotic resistant genes be transferred from nonpathogenic bacteria to human pathogenic strains?" Brooks et al. (2004) and Brooks et al. (2007) concluded that Class B biosolids had an equal or lower incidence of antibiotic resistant bacteria compared to unamended soil. The NRC (2002) did not "believe that land-applied biosolids have any substantial potential to alter the prevalence of antibiotic resistance among pathogenic organisms."

Infectivity

Gerba and Smith (2005) describe broad risk assessment principles for land application of wastes based on a quick review of the literature, as well as their own experience and expertise. They note that information on infectivity of enteric pathogens is available from many human feeding or inhalation studies.

Dose-response data suggest that a threshold infectious dose does not exist for enteric pathogens (Gerba and Smith, 2005). Infectivity of enteric viruses is greater than infectivity of enteric bacteria. Of known human enteric viruses, rotavirus is the most

infectious, causing 10-15% of those ingesting the virus to become infected. Half of the people infected with an enteric pathogen become ill. Mortality is typically less than 1%, but greater for infants, young children, the elderly and immunocompromised people (Gerba and Smith, 2005).

Nwachuku and Gerba (2004) address the susceptibility of children to pathogens, including increased sensitivity and increased exposure. Reasons that children are at greater potential risk from pathogens in biosolids are

- immature immune system;
- intestinal mucosa more permeable to water;
- proportionally less extracellular fluid than adults;
- physiological deficiency in IgA;
- reduced stomach acid and pepsin secretion.

For example, children appear to be the most sensitive population to enteroviruses. Studies have not been conducted to estimate relative infectivity of enteric pathogens for children and adults. However, reduced stomach acid and pepsin secretion could make children more likely to be infected than adults for a given dose.

Disease Risk

Empirical studies of biosolids do not estimate disease risk. However, risks of disease might be assumed to be 10% that of infectious risk, though this quantity varies with microorganism (Haas et al., 1999).

Dynamic Risk Model

Eisenberg et al. (2004) developed a deterministic, dynamic model for estimating risks from pathogens in biosolids. In addition to infectivity, their model considered person-to-person transmission, immunity, asymptomatic infection and incubation period. The model contains six disease states: (1) susceptible state, (2) exposed state (asymptomatic and infectious), (3) carrier state 1 (asymptomatic but infectious), (4) diseased state, (5) carrier state 2 (previously symptomatic, now asymptomatic and infectious) and (6) protected state (postinfectious and noninfectious and some level of immunity). Processes that were not accounted for include climate, behavior and various environmental factors that are not well understood. Three types of risks were estimated: individual-level single event risk, individual-level annual risk and population level attributable risk (Eisenberg et al., 2006). The model was demonstrated in a case study involving the direct ingestion of enterovirus. Sensitivity analysis of simulations in the case study showed that the four most important factors in determining the risk attributable to biosolids were (1) the relative contribution of biosolids toward exposure, relative to other pathways; (2) the rate of pathogen shedding by infectious people; (3) the rate of person-to-person transmission and (4) immunity. Risk attributable to biosolids was “low” if the rate of pathogen shedding was relatively high or low or if person-to-person transmission was relatively “high.” These were not necessarily intuitive results. The simulations resulted in a decision tree for classifying risk associated with biosolids as high or low.

EXPOSURE ASSUMPTIONS

U.S. EPA does not have standard exposure factors for use in risk assessments of pathogens in biosolids. Risk assessment results described above are highly dependent on human exposure factors, and these vary from study to study. For example, because human transmission of aerosols containing *Salmonella* has not been demonstrated, researchers make different assumptions about the percentage of inhaled particles that would be ingested. Pepper et al. (2006) describe studies that use 10%, and Brooks et al. (2005b) uses 50%.

Very little information is available that would allow us to compare the relative importance of different exposure pathways. Academic studies tend to emphasize a single exposure pathway rather than a comparison of multiple pathways. Many studies have found low risk. For example, a British study by Gale (2005b) concluded that risk to human health from consumption of vegetation crops contaminated with pathogens in biosolids is low. Moreover, a study of bioaerosols in Arizona found that risk of infection of residents from bioaerosols generated during land application of biosolids was rather negligible at 10 km, though if residents were assumed to reside closer, estimated risks would have been higher (Brooks et al., 2005b; Pepper et al., 2006). Based on a review of the literature, Pepper et al. (2006) conclude that “groundwater contamination from land-applied biosolids does not appear to be likely.” Moreover, it is argued that regrowth of pathogens in biosolids-amended soil may be ignored because of the biological competition in Class B biosolids (Pepper et al., 2006; Zaleski et al., 2005a,b). However, insufficient information is available to ignore particular exposure pathways at all sites.

CAUSAL ANALYSIS

"Causal association between biosolids exposures and adverse health outcomes has not been documented" (NRC, 2002). Lewis et al. (2002) recorded symptoms reported by 48 residents near 10 biosolids application sites in the U.S. and Canada. The wide range of symptoms included various combinations of coughing, burning eyes, sore throat, burning lungs, headache, congestion, difficulty breathing, flu-like symptoms, fever, nausea/vomiting, diarrhea, sinusitis, staphylococcal infection, pneumonia, skin rash, nosebleed and fatigue. The researchers did not establish cause and effect between biosolids and reported adverse effects. They speculated that chemical contaminants in biosolids might irritate the skin and mucous membranes and thus increase pathogen host susceptibility (Lewis et al., 2002).

Dorn et al. (1985) conducted a health effects study of 47 biosolids application sites (annual applications) and 46 control sites on farms in Ohio. Estimated risks of respiratory illness, digestive problems or other general symptoms did not differ between biosolids and non-biosolids farms. The authors cautioned readers when considering the results in the context of larger acreages, higher application rates or biosolids containing larger concentrations of pathogens.

NRC (2002) summarized studies of sewer workers and others exposed to raw sewage to identify potential hazards from biosolids. The committee also summarized a survey study in which workers who loaded, unloaded and applied Class B biosolids had a history of gastrointestinal illness. However, it was later determined that the biosolids did not meet Class B requirements.

Simmonds et al. (2005) describe the difficulties of conducting an epidemiological study of biosolids exposure. Few people who are exposed are expected to become infected, and even fewer to manifest symptoms of disease. Also, various symptoms may be associated with one pathogen, and various pathogens can cause similar symptoms.

A recent abstract indicates that a health effects study of biosolids exposure is underway (Heaney et al., 2007).